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"WELL considered and conservative measures will likewise be proposed which will attempt to give to industrial workers of the country a more fair wage return, prevent cut-throat competition and unduly long hours for labor, and at the same time to encourage each industry to prevent over-production.....

"GOVERNMENT ought to have the right and will have the right, after surveying and planning for an industry, to prevent, with the assistance of the overwhelming majority of that industry, unfair practice and to enforce this agreement by the authority of government."

(From the May 7 radio address of Franklin D. Roosevelt.)

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PUTTING THE BAN ON POLITICS

Members of the Washington State Veterinary Medical Association have prevailed upon the new Democratic Governor, Clarence D. Martin, to retain in office Dr. Robert Prior as Director of Dairy and Live Stock, which office is equivalent to that of State Veterinarian. As Washington was one of the states that changed from a Republican to a Democratic organization, as a result of the 1932 elections, the significance of Governor Martin's act will be appreciated.

On October 21, 1932, the Western Branch of the Washington State Veterinary Medical Association met in Tacoma and adopted a resolution, copies of which were forwarded to both the Republican and Democratic candidates for Governor, asking for the retention of Dr. Prior. The resolution pointed out that a neighboring state—Oregon—had retained Dr. W. H. Lytle in a similar office for 25 years under both major political parties, to the distinct advantage of Oregon's live stock industry.

The veterinary profession in some of the states that seem unable to divorce animal disease control from party politics might take a leaf out of Washington's book. Dr. Prior's administration of his office must have been satisfactory to the veterinarians of Washington or they would not have taken the action they did.

A SHORTSIGHTED POLICY

A member of the profession recently claimed that he had located the "meanest" veterinarian. When asked what particular qualifications won this rather undesirable appellation, our friend replied that the "meanness" consisted in buying anti-hog cholera serum from a "farmer" serum company, because he could buy it "cheaper" from this "outfit" than he could from any of the companies that restrict sales to veterinarians. Now then, was this veterinarian "mean" or was he shortsighted and unappreciative? Did he or anybody else ever hear of one of these "farmer" serum companies doing anything to help the veterinary profession? No! Even if he is one of the class who never attend veterinary meetings or who never subscribe to a veterinary journal, he hardly could claim to be ignorant of what some of our commercial concerns have done in a constructive way for the veterinary profession.

A veterinarian who patronizes one of these "front door-side door" serum companies is giving comfort to the enemy. These concerns are not interested in having hog cholera controlled. They wax fat on farmer vaccination, because virus promiscuously distributed means more hog cholera. They do not want shipments of virus restricted. That hurts their business. One of the companies referred to has even gone as far as to advertise a table of dosage for serum that is regarded as too low for safety by careful, conscientious veterinarians, who want to do the right thing by their clients—and their clients' neighbors, too.

Just suppose that you, as an ethical veterinarian, were to receive a letter one of these days, from the company from which you have been purchasing your serum—one of the "single standard" concerns—advising you that on and after a certain date the company would discontinue its policy of restricting sales to veterinarians, and would start selling direct to farmers, farm bureaus and county agents. How would you feel about it? And then suppose others would follow. It's possible. Everybody has seen things happen the past year that we all believed to be impossible. And we are going to see more things happen before the present "mess" is cleaned up.

If there is any moral to all this, it is to be "pound wise" rather than "penny foolish." Don't run the danger of being classified as "mean," when "shortsighted" would be more nearly correct. Think it over. And think hard.

Chicago has over 200 miles of bridle paths.

THREE NEW STATE VETERINARIANS

Dr. J. Leonard Axby (Chi. '03), of Lawrenceburg, Ind., succeeded Dr. Frank H. Brown (Ind. '10), as State Veterinarian of Indiana, on May first. Dr. Axby has been an outstanding figure in the Hoosier State for many years. He served as mayor of his city from 1910 to 1914, and as State Representative from Dearborn and Ohio counties in the 1917 and 1919 legislatures. He has held many important offices and committee assignments in the Indiana State Veterinary Medical Association and the American Veterinary Medical Association.

During the 1933 annual meeting of the Tennessee Veterinary Medical Association, held in Nashville, in January, the organization endorsed two members for the position of State Veterinarian, namely, the incumbent, Dr. J. M. Jones (McK. '12), and Dr. A. C. Topmiller (McK. '07). On account of the change in the state administration and a desire upon the part of the new governor to organize an entirely new official family, Dr. Topmiller was given preference over Dr. Jones and succeeded him on May 15. From this it will be seen that the governor of Tennessee respected the wishes of the veterinary profession in his State.

Dr. H. A. Seidell (Iowa '18), of Fort Dodge, Iowa, has been appointed chief of the Division of Animal Health by Hon. Ray Murray, secretary of the Iowa Department of Agriculture. Dr. Seidell succeeds the late Dr. N. O. Bevins, who died suddenly on May 11. He was assistant state veterinarian in the Eighth District prior to his appointment as State Veterinarian.

The following states have changed their chief veterinary sanitary control officials during the past six months: Arizona, Arkansas, California, Indiana, Iowa, North Dakota, South Dakota, Tennessee and Texas.

EXECUTIVE BOARD ELECTIONS

Polls for the Executive Board elections now in progress in Districts 2 (Pennsylvania, New Jersey and Delaware) and 3 (Illinois, Indiana and Wisconsin) will be closed on June 14. Although the balloting has been heavy, quite a few members entitled to vote have not yet done so. Members who have not paid their 1933 dues have not received ballots. However, in such cases ballots will be mailed on receipt of remittances up to

June 12. Results of the elections will be announced in the July issue of the JOURNAL.

DOCTOR MOHLER AGAIN HONORED

At a meeting held in Paris recently, the Permanent Committee of the International Veterinary Congress elected Dr. John R. Mohler, chief of the United States Bureau of Animal Industry, vice-president, to succeed Dr. Emmanuel Leclainche, chief of the Government Veterinary Services in Paris. Dr. Leclainche was elected president to succeed the eminent bacteriologist, Dr. Francis von Hutyra, Rector of the Royal Hungarian Veterinary High School, Budapest, Hungary, who resigned the office on account of serious illness. Dr. Mohler also is treasurer of the Organizing Committee of the Twelfth International Veterinary Congress.

APPLICATIONS FOR MEMBERSHIP

(See January, 1933, JOURNAL)

FIRST LISTING

AHRENS, GEORGE A. 6351 Normal Ave., Chicago, Ill.
D. V. M., Indiana Veterinary College, 1916
Vouchers: Edward Himsel and G. E. Totten.

BRADHAM, GUY E. Cisne, Ill.
D. V. M., Indiana Veterinary College, 1911
Vouchers: W. H. Welch and A. E. Dickerson.

CORLISS, JAMES R. State House, Augusta, Me.
D. V. M., Kansas City Veterinary College, 1915
Vouchers: M. E. Maddocks and L. K. Green.

HELD, VINCENT 1504 Oregon Ave., Klamath Falls, Ore.
D. V. M., Iowa State College, 1931
Vouchers: W. H. Lytle and Chas. H. Seagraves.

MEGINNIS, PAUL J. 6421 Harper Ave., Chicago, Ill.
D. V. M., Iowa State College, 1931
Vouchers: Edward Himsel and H. B. Raffensperger.

Applications Pending

SECOND LISTING

(See May, 1933, JOURNAL)

Davenport, Roy F., Duncannon, Pa.
Larsen, Osborne G., Newton, Utah.
Smead, Harold L., 274 Leyden Road, Greenfield, Mass.

The amount which should accompany an application filed this month is $7.91, which covers membership fee and dues to January 1, 1934, including subscription to the JOURNAL.

COMING VETERINARY MEETINGS

New York City, Veterinary Medical Association of. Academy of Medicine, 5th Ave. and 103rd St., New York, N. Y. June 7, 1933. Dr. John E. Crawford, Secretary, 708 Beach 19th St., Far Rockaway, Long Island, N. Y.

San Diego-Imperial Veterinary Medical Association. San Diego, Calif. June 7, 1933. Dr. A. P. Immenschuh, Secretary, Santee, Calif.

Tulsa County Veterinary Association. Tulsa, Okla. June 8, 1933. Dr. J. M. Higgins, Secretary, 3305 E. 11th St., Tulsa, Okla.

East Tennessee Veterinary Medical Society. White Surgical Supply Co., Knoxville, Tenn. June 10, 1933. Dr. R. E. Baker, Secretary, Morristown, Tenn.

American Association of Medical Milk Commissions. Hotel Plankinton, Milwaukee, Wis. June 11-13, 1933. Dr. Harris Moak, Secretary, 360 Park Place, Brooklyn, N. Y.

Interstate Veterinary Medical Association. Elks Building, Omaha, Neb. June 12, 1933. Dr. G. L. Taylor, Secretary, Plattsmouth, Neb.

Chicago Veterinary Medical Society. Hotel LaSalle, Chicago, Ill. June 13, 1933. Dr. O. Norling-Christensen, Secretary, 1904 W. North Ave., Chicago, Ill.

Southeastern Michigan Veterinary Medical Association. Detroit, Mich. June 14, 1933. Dr. A. S. Schlingman, Secretary, Parke, Davis & Co., Detroit, Mich.

Eastern Iowa Veterinary Association, Inc. Mechanicsville, Iowa. June 15, 1933. Dr. Iva Dunn, Secretary, Atkins, Iowa.

American Association for the Advancement of Science. Chicago, Ill. June 19-30, 1933. Dr. Henry B. Ward, Secretary, Smithsonian Institution Building, Washington, D. C.

California State Veterinary Medical Association. San Diego, Calif. June 19-21, 1933. Dr. Geo. M. Simmons, Secretary, 1386 Golden Gate Ave., San Francisco, Calif.

Oklahoma Veterinary Medical Association. Fort Reno, Okla. June 19-20, 1933. Dr. C. H. Fauks, Secretary, 1719 S. W. 15th St., Oklahoma City, Okla.

Texas, State Veterinary Medical Association of. Fort Worth, Tex. June 19-21, 1933. Dr. D. Pearce, Secretary, Box 335, Leonard, Tex.

Kansas City Veterinary Association. Baltimore Hotel, Kansas City, Mo. June 20, 1933. Dr. J. D. Ray, Secretary, 1103 E. 47th St., Kansas City, Mo.

Southern California Veterinary Medical Association. Chamber of Commerce Building, Los Angeles, Calif. June 21, 1933. Dr. T. G. Beard, Secretary, 3684 Beverly Blvd., Los Angeles, Calif.

Maryland State Veterinary Medical Association. College Park, Md. June 22-23, 1933. Dr. E. M. Pickens, Secretary, College Park, Md.

Michigan State Veterinary Medical Association. Lansing, Mich. June 27-28, 1933. Dr. E. K. Sales, Secretary, 535 Forest St., East Lansing, Mich.

North Carolina State Veterinary Medical Association. Hendersonville, N. C. June 28-29, 1933. Dr. J. Howard Brown, Secretary, Tarboro, N. C.

New York State Veterinary Medical Society. Albany, N. Y. June 29-30, 1933. Dr. J. G. Wills, Secretary, Box 751, Albany, N. Y.

Minnesota State Veterinary Medical Society and University of Minnesota Short Course for Veterinarians. University Farm, Saint Paul, Minn. July 6-7, 1933. Dr. C. P. Fitch, Secretary, University Farm, Saint Paul, Minn.

Washington State Veterinary Medical Association. Olympia, Wash. July 10-12, 1933. Dr. Clifford Ackley, Secretary, Winlock, Wash.

Kentucky Veterinary Medical Association. Brown Hotel, Louisville, Ky. July 12-13, 1933. Dr. E. A. Caslick, Secretary, Paris, Ky.

Maine Veterinary Medical Association. State House, Augusta, Me. July 12, 1933. Dr. L. E. Maddocks, Secretary, R. F. D. 2, Augusta, Me.

Vermont Veterinary Medical Association. Brandon, Vt. July 13-14, 1933. Dr. G. N. Welch, Secretary, 43 Union St., Northfield, Vt.

Virginia State Veterinary Medical Association. Fredericksburg, Va. July 13-14, 1933. Dr. I. D. Wilson, Secretary, Virginia Polytechnic Institute, Blacksburg, Va.

Northeastern Indiana Veterinary Medical Association. Tri-Lakes, Ind. July 16, 1933. Dr. H. W. Demsey, Secretary, Huntington, Ind.

Missouri Veterinary Medical Association. Joint meeting with Saint Louis District Veterinary Medical Association. Marquette Hotel, Saint Louis, Mo. July 18-19, 1933. Dr. J. D. Ray, Secretary, 1103 E. 47th St., Kansas City, Mo.

South Carolina Association of Veterinarians. Aiken, S. C. July 18-19, 1933. Dr. G. J. Lawhon, Secretary, Hartsville, S. C.

New Jersey Veterinary Medical Association. Hotel Madison, Atlantic City, N. J. July 20-21, 1933. Dr. John G. Hardenbergh, Secretary, c/o Walker-Gordon Lab. Co., Plainsboro, N. J.

Montana Veterinary Medical Association. Bozeman, Mont. July 27-28, 1933. Dr. Hadleigh Marsh, Secretary, Agricultural Experiment Station, Bozeman, Mont.

Saskatchewan, Veterinary Association of. Regina, Sask. July 27-28, 1933. Dr. Norman Wright, Secretary, 137 20th St., West, Saskatoon, Sask.

Poultry Science Association. Michigan State College, East Lansing, Mich. August 2-5, 1933. Professor C. G. Card, General Chairman, East Lansing, Mich.

American Veterinary Medical Association. Palmer House, Chicago, Ill. August 14-18, 1933. Dr. H. Preston Hoskins, Secretary, 1230 W. Washington Blvd., Chicago, Ill.

Fifth World Poultry Congress. Trajan's Markets, Rome, Italy. September 6-15, 1933. Comitato del V Congresso Mondiale di Pollicoltura, Ministero dell' Agricoltura delle Foreste, Roma, Italy.

STATE BOARD EXAMINATIONS

Massachusetts Board of Registration in Veterinary Medicine. Department of Civil Service & Registration, Boston, Mass. July 6-7, 1933. Dr. E. W. Babson, Secretary, 343 Washington St., Gloucester, Mass.

Oklahoma State Board of Veterinary Medical Examiners. City Hall, El Reno, Okla. June 21, 1933. Dr. Walter H. Martin, Secretary, 101 S. Evans St., El Reno, Okla.

Iowa Veterinary Medical Examining Board. State House, Des Moines, Iowa. June 13-14, 1933. Candidates for examination must be in the office of the Division of Animal Industry not later than 9:00 a. m., June 13. Examination fee, $25.00. Dr. Peter Malcolm, Secretary, State House, Des Moines, Iowa.

THE CORRELATION BETWEEN CERTAIN PROPERTIES OF MILK AND THE TYPE OF INFLAMMATION IN ACUTE MASTITIS*

By RALPH B. LITTLE *and* F. S. JONES
Department of Animal and Plant Pathology
Rockefeller Institute for Medical Research
Princeton, N. J.

Since the time it was first noted that udder abnormality was indicated by changes in the milk, many observations dealing with such variations have been published. In the main, however, the discussion has centered about the observed abnormalities as an aid to the diagnosis of udder derangement, largely for the purpose of excluding milk of infected individuals from the general supply. For the most part the idea of combining the findings with the clinical pathology of the udder has been scarcely considered.

Probably the first significant difference between normal and abnormal milk noted was the relatively small number of leucocytes present in the former compared with enormous number found in milks obtained from inflamed udders. Storch,[1] as early as 1889, noted that the milk from inflamed udders was more alkaline than normal, but due to the unreliability of the methods then in use, little significance was attached to changes in the reaction of milk. With the development of more reliable indicators, slight changes in pH were detected readily and since Baker and Van Slyke[2] showed that milk from inflamed udders tends to be more alkaline than the normal secretion, the method has been utilized as an aid in detecting udder inflammation. Many investigators noted that milk from certain cases of mastitis coagulated when boiled, so that the appearance of certain kinds of coagulable proteins became of significance in the recognition of the disease. Others have commented on the diminution in the quantity of lactose during udder inflammation. The appearance of chlorids in increasing quantities and the appearance of histidin have been recorded by Sjollema and Van der Zende.[3]

The presence of leucocytes, the changes in the pH of the milk, and the increase of certain proteins in the whey seemed to us to indicate certain types of reaction to bacterial invasion which may occur within the udder, and with these reactions in mind we studied the milk from a number of cases of acute udder dis-

*Presented at the sixty-ninth annual meeting of the American Veterinary Medical Association, Atlanta, Ga., August 23-26, 1932.

ease in a large dairy herd. The cases were unselected. Many were first attacks, others had been affected before, but all the data were obtained from milk drawn early in the particular attack, usually within a few hours of clinical onset.

Material and Methods

Milk from 15 cases was drawn directly into sterile bottles from all four quarters, a few hours after clinical symptoms had developed. It was brought to the laboratory and examined at once.

For bacteriological examination a portion of each sample was diluted in 10 parts of NaCl solution and 0.5 cc plated with 0.5 cc of defibrinated horse blood and 12 cc of 2 per cent veal infusion agar; in addition the sediment from 10 cc of milk usually was streaked on the surface of poured agar plates containing horse blood. The bacteriological examinations were completed after incubation at 38° C. for 48 hours.

Leucocytes were counted by means of the Breed and Brew[4] method. The pH was determined colorimetrically and the volumetric measure of the whey proteins found by the procedure devised by us.[5]

Inasmuch as the data obtained from the 60 separate examinations were too voluminous to be published in their entirety, they have been summarized in table I.

Table I—*Summary of the findings in acute mastitis.*

Quarters Invaded by Potential Pathogens	Quarters the Milk from Which Contained 1 Million or More Leucocytes per cc	Samples Having an Abnormal Reaction		Samples Containing Excessive Quantities of Whey Protein
		pH 6.8-7.2	pH 5.0-6.0	
47	46	30	3	40

The summary of the data in table I indicates that the milk from 47 of 60 quarters of 15 cows contained streptococci or hemolytic staphylococci in sufficient numbers to account for the inflammation. The results of the examination of the milk from the 13 normal quarters have been omitted, since such milk appeared normal.

It is of interest to note that 46 of the 47 milks from abnormal udders contained leucocytes in excessive numbers. Increase in

whey protein, found in 40 samples, was next in frequency. In 33 of the 47 samples there were changes in the pH, three were definitely more acid and 30 more alkaline than the normal secretion.

If the correlation between various abnormal factors be carried further, as in table II, where the whey protein is compared with pH, certain facts are brought out. In this and subsequent tables the results of all 60 examinations are given.

In table II the data indicate in general that milks having an abnormal pH are apt to contain more whey protein than normal milk, but the correlation is not absolute. In three instances there was no increase in protein although the reaction was slightly

TABLE II—*Precipitable whey protein as determined volumetrically, compared with pH.*

	pH 5.0–6.0	pH 6.6	pH 6.8–7.0	pH 7.2–7.4
Samples showing normal quantities of whey proteins	0	16	2	1
Samples showing abnormal quantities of whey proteins	3	11	14	13

more alkaline. In eleven milks the reverse was true, the milk was normal in regard to pH but contained whey proteins in excess of the normal.

Baker and Breed[6] compared the reaction of milk to the number of leucocytes and found in general a close correlation, samples containing large numbers of leucocytes being most alkaline. Where relatively few leucocytes were found, the reaction was that of the normal milk. For completeness, we have considered these comparisons in our series, and the results have been recorded in table III.

Much the same type of comparison exists between the number of leucocytes and pH as between whey protein and pH. Samples containing large numbers of leucocytes are apt to show changes in pH although certain exceptions are noted. If, for purposes of argument, more than 1,000,000 leucocytes per cc are regarded as indicative of abnormality, then in 16 instances such milk was normal in reaction (pH 6.6). In three instances where the leucocytes were below 1,000,000 per cc, the samples were a little more alkaline than normal.

TABLE III—*Leucocytes and the pH of milk.*

LEUCOCYTES PER CC	pH 6.6	pH 6.8–7.2	pH 5.0–6.0
Below 1 million	16	3	0
Between 1 and 2 million	2	2	0
Between 2 and 3 million	2	0	1
Between 3 and 4 million	3	0	0
Between 4 and 5 million	0	1	0
Between 5 and 6 million	1	0	0
Between 8 and 9 million	0	1	0
Above 9 million	3	23	2

If comparisons are carried still further and the number of leucocytes correlated with whey proteins, as in table IV, similar correlations are obtained.

Much the same type of correlation exists between leucocytes and whey protein as between leucocytes and pH or between pH and protein. In milks where the leucocytes were above 4,000,000 per cc, only three of the 31 samples showed no increase in whey

TABLE IV—*Comparison of whey protein and leucocytes.*

LEUCOCYTES PER CC	SAMPLES FAILING TO SHOW INCREASE IN WHEY PROTEIN	SAMPLES SHOWING WHEY PROTEIN INCREASE
Below 1 million	13	6
Between 1 and 2 million	2	2
Between 2 and 3 million	1	2
Between 3 and 4 million	1	2
Between 4 and 5 million	0	1
Between 5 and 6 million	0	1
Between 8 and 9 million	0	1
Above 9 million	3	25

protein, while 28 did show an increase. Where the leucocytes numbered 1,000,000 or under per cc, about one-third of the specimens had more whey protein than normal. Of the 10 containing between 1,000,000 and 4,000,000 leucocytes per cc, six showed no increase in protein.

Since in general there is no sharp relationship between the various factors and in order to develop our argument further, it seems best to cite certain specific findings, shown in table V.

The seven examples cited, chosen from the 15 spontaneous cases of mastitis, emphasize certain facts. Thus, in 5474 and

TABLE V—*Observed variations in selected cases of mastitis.*

CASE	LEUCOCYTES PER CC	VOLUME OF WHEY PROTEIN PER CC (MM)	pH
5474	51,000,000	51.6	7.2
6440	Innumerable	74.4	5.0
7243	Innumerable	8.1	6.6
K 96	81,000,000	6.3	7.0
8048	25,000,000	19.2	6.6
K 96 R. F.	1,500,000	19.2	7.2
8048 L. H.	3,000,000	16.8	6.6

6440 there occurred a marked leucocytosis with relatively large quantities of whey protein and sharp changes in the pH. Case 7243 may be regarded as distinctly purulent in character, since only leucocytosis without change of pH or protein was noted. Case K 96 illustrates leucocytosis and increased alkalinity without increase of whey protein. In sample 8048 there was a distinct increase in whey protein and the milk was purulent but the pH that of normal milk. Cases K 96 R. F. and 8048 L. H. contained leucocytes in more moderate numbers but both had more whey protein than the normal, the reaction of one being pH 7.2 and the other normal (6.6).

In another paper we discussed the character of the proteins found during udder inflammation and correlated them with blood proteins. Since this phase of the observations has already been discussed, all reference to these data has been omitted from the present paper.

DISCUSSION

In general it may be said that a fair degree of correlation exists between bacterial infection and udder response. In most cases leucocytosis may be regarded as the most frequent finding, but in a large proportion of cases the milk contains excessive quantities of protein in the whey fraction. The alterations in pH are, on the whole, less frequent than was expected. Since there is no hard and fast correlation between certain of the abnormalities noted in the milks, it is possible that our findings are indicative of individual variations of pathological reactions occurring within the udder. For example, case 7243, cited in table V, appears to illustrate an entirely cellular type of reaction to udder invasion by microörganisms. Here the milk was purulent, nevertheless, its reaction was normal (pH 6.6) and there was no increase in the proteins of the whey fraction. Case 5474 might

be cited as a more usual reactive process in the acute disease where there was a well-marked leucocytic response accompanied by a marked increase in whey protein and a distinct increase in alkalinity. Case 6440 differs in that the milk was distinctly acid, a fact that will be commented upon later. Between the wholly purulent and the sero-purulent types, intergrades exist. For example, the milk may be purulent but contain only normal quantities of whey protein and be much more alkaline than normal, as in case K 96. Purulence may be noted, with increase of whey protein but without changes in reaction, as in case 8048. The last two examples cited in table V are even more atypical, in that there is relatively little leucocytosis, but both show increase in the whey proteins although in one case only (K 96 R. F.) was there a change in pH.

The udder then may react differently under various conditions. The evidence indicates that in general, during an acute attack, not only is there leucocytosis but an increase in alkali and blood protein. From our observations this might be considered the most frequent process. In other instances the exudate may be entirely leucocytic without change in reaction or increase in whey protein. At times leucocytosis is pronounced and whey protein may be increased but the pH remain that of normal milk. Again, the milk may be alkaline and serous without much cellular reaction, or it may be of a slight purulence, contain considerable whey protein and the pH remain 6.6. These findings seem to indicate to a certain extent the severity of the inflammatory processes. Thus, where the milk is purulent but contains only small quantities of whey protein and its pH is that of normal milk, the process is less severe and probably superficial. In the cases where in addition to leucocytosis there is a sharp rise in the whey protein and a corresponding rise in pH, a tissue destruction and vascular derangement is indicated. From our observations we regard the augmentation of whey protein as originating in the blood, since we have shown that much of this fraction may be precipitated by half-saturated ammonium sulfate. We regard it as globulin and by means of precipitin tests have correlated it with blood globulin. The udder capillaries then, under more severe inflammatory conditions, are permeable to blood proteins. Although we have no data which directly bear on the cause of the increased alkalinity under these conditions, nevertheless, the fact that the capillaries are permeable to proteins indicates that the same structures may permit the passage of blood alkali. In udders where the inflammation is less severe, aside from leucocy-

tosis, there may be only a moderate permeability to blood protein, and whereas a relatively large passage of base may occur, nevertheless, the buffer effect of the milk is sufficient to hold the pH at the normal level. The same sort of explanation may be given where the whey protein is moderately increased but the pH shifted to the alkaline side. Here the permeability may be marked for salts so that they enter the milk in sufficient quantities to change the pH. In certain experiments it has been found that vascular permeability, as measured by volume of whey protein, persists for several days during convalescence when the number of streptococci has sharply declined and the leucocytic reaction is on the wane. This might explain the observations when the number of leucocytes was relatively small with the milk still containing considerable blood protein.

The results in general are in accordance with those of Baker and Breed,[6] who showed that a correlation usually existed between the number of leucocytes and the pH. They explained the divergence in certain instances to the fermentation of lactose by the streptococci, thus tending to neutralize the effect of blood alkali. Stabelforth[7] argued that during inflammation there is actual damage to the alveolar membrane, permitting, under severe conditions, the passage of blood-serum or lymph and, under milder processes, only blood bases. Ernst[8] pointed out that injury to alveolar structure occurred during mastitis and suggested that the amount of serum found in the milk was proportional to such injury.

Abnormal acidity of certain samples of milk is of interest and has been encountered in a few instances. Little is known concerning the frequency of the phenomenon, but certainly it does not appear so often as our protocols indicate. Baker and Breed evidently encountered it and suggested that it might result from the fermentation of carbohydrate by the streptococcus. Under certain conditions this seems to be a plausible explanation, but the phenomenon must be studied further before more definite conclusions may be reached.

SUMMARY

The milk from acute cases of mastitis has been studied from the standpoint of its leucocyte content, the quantity of whey proteins as determined volumetrically, and the pH. These factors have been correlated with udder infection. In general there is a close correlation between bacterial invasion and leucocytosis. In many cases the whey proteins are measurably increased in volume and in many instances the reaction of the milk is altered,

usually toward the alkaline side. The manifested changes in the milk have been related to the severity and character of the inflammatory process.

REFERENCES

[1]Storch, V.: Jahresb. Thierchen., xix (1889), p. 157.
[2]Baker, J. C., and Van Slyke, L. L.: Jour. Biol. Chem., xl (1919), p. 357.
[3]Sjollema, B., and Van der Zende, J. E.: Jour. Biol. Chem., liii (1922), p. 513.
[4]Breed, R. S., and Brew, J. D.: N. Y. Agr. Exp. Sta. Bul. 149 (1916).
[5]Jones, F. S., and Little, R. B.: Jour. Dairy Sci., xvi (1933), p. 101.
[6]Baker, J. C., and Breed, R. S.: Jour. Biol. Chem. xliii (1920), p. 221.
[7]Stabelforth, A. W.: Jour Comp. Path. & Therap., xliii (1930), p. 22.
[8]Ernst, W.: Gesendheitsompte der Milchhygiene für Tierartz. (Stuttgart, 1913.) viii, p. 14.

Airplane Service to Chicago

Airplane service to Chicago from all parts of the United States is now available for those who desire to utilize this form of transportation to come to the A. V. M. A. convention in August. The one-way fares applying on air lines are now equivalent to approximately rail plus Pullman rates, with a ten per cent reduction on round trips.

Frequently a combination of rail-plane service offers speedy travel for those living in cities not directly on air lines. There is practically no city in the United States more distant from Chicago than 24 hours by airplane.

Illustrative of the speed with which travelers may come to Chicago if pressed for time, by using tri-motored passenger planes of lines operating daily schedules to Chicago, are the following examples:

City	*Time (Hours)*	*City*	*Time (Hours)*
Atlanta, Ga.....	7	New York, N. Y.....	6
Bismarck, N. D.....	12	Oklahoma City, Okla....	8
Boston, Mass.....	10	Omaha, Neb.....	4
Cincinnati, Ohio.....	3	Philadelphia, Pa.....	8
Cleveland, Ohio.....	3	Portland, Ore.....	24
Dallas, Tex.....	10	Saint Louis, Mo.....	3
Denver, Colo.....	12	Salt Lake City, Utah....	15
Detroit, Mich.....	3	San Francisco, Calif.....	22
Kansas City, Mo.....	4	Washington, D. C.....	7
Los Angeles, Calif....	22	Wichita, Kan.....	6
Minneapolis, Minn.....	4	Winnipeg, Man.....	12
New Orleans, La.....	10		

During 1932, the number of live stock on farms in the United States increased 2 per cent over the previous year, but the value decreased 17 per cent, reports the U. S. Department of Agriculture.

A STUDY OF BOVINE COCCIDIOSIS, II*

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Introduction

In a previous paper,[1] one of us pointed out that bovine coccidiosis is one of the most widely spread parasitic diseases. It has been reported from all parts of the world and from all sections of the United States. For the most part the disease is so mild in character that symptoms escape notice. Not infrequently, however, acute outbreaks occur, causing many deaths or great loss of flesh among the survivors.

Experimental

PURPOSE OF STUDY

The reports in the literature within the last ten years have pointed out the prevalence and economic importance of bovine coccidiosis. The work of Tyzzer[2] with chicken coccidia has stimulated investigations and the study of bovine coccidiosis by Wilson[1] has opened up new fields for research. The knowledge existing previous to these two reports was based upon field observations and inconclusive laboratory experimentation. Great advancement has been made during the last three years in the study of the biology of the species of Eimeria affecting the chicken. A study of the disease in cattle is much more difficult because a large number of animals cannot be kept for laboratory experimentation under ideal conditions, except at great expense. For this reason, advancement in the study of bovine coccidiosis will be slow and it will be necessary to confine investigation and experimentation to certain definite phases of the disease. This investigation has been limited to a study of the physiologic characteristics of the species of Eimeria affecting cattle, and to a general consideration of the disease.

MATERIALS AND METHODS

During the course of this work 19 calves, one cow and a number of white rats have been used. The calves, either Jersey or Holstein, were obtained when they were less than a week old, and

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†Resigned June 1, 1932.

were kept under observation before being placed on experiment. They were confined in a large, well-ventilated and well-heated room which was neither rat-proof nor fly-proof. Their quarters were separate cages made of steel mesh, with solid steel walls, which gave them no chance to communicate with each other. The cages and their removable wooden floors were cleaned daily with a hose. The cement floor of the room was equipped with a drain to carry off all fecal material. The feeding troughs were hooked to the doors, and were removed after feeding to prevent contamination with fecal matter. Before an animal was placed on experiment, the cages and floors were cleaned thoroughly and disinfected with high pressure steam. The young animals were fed milk, and later chopped alfalfa hay and a dairy ration, in the proper amounts. Green grass was given regularly, except during the winter months. This ration gave good results; the calves grew and gained as much as could be expected in close confinement. The milk was pasteurized and fed in individual pails, which were washed and steamed after each feeding. No attempt was made to sterilize the feed because it was considered impractical and the possibilities of accidental infection under proper handling were believed to be almost negative. Two or three non-infected susceptible control animals were kept at all times in the same room with the infected calves. At no time during the course of the experiment was there a case of accidental infection in the control calves.

Daily fecal examinations were made on all calves for at least two weeks after they were obtained. Animals that were not being used on the experiment were examined three times a week. Only two calves were found to be passing coccidia at the time they were secured. These two passed an occasional coccidium, but soon became negative. The sugar flotation method, described by Benbrook,[3] was followed in making fecal examinations. Fecal samples were obtained for all examinations by dilating the anus and rectum, which procedure insured fresh representative samples.

The oöcysts were incubated in either 1.5 per cent potassium dichromate or 2 per cent copper sulfate solution in a room ranging in temperature from 20 to 25° C. Half-gallon jars containing three pints of culture material were used to sporulate the oöcysts. The cultures were aerated once or twice a day, from three to five minutes, by forcing air into the bottom of the solution with a pressure bulb. Pint milk bottles with cork stoppers to prevent evaporation were used when there was not a large

amount of material to be sporulated. The bottles were shaken two or three times daily, and the corks were removed at intervals to change the air. Flat-bottomed porcelain pans were used when maximum sporulation was desired. The cultures were prepared by adding equal amounts of copper sulfate or potassium dichromate solution to a suspension of fecal material which had been screened to remove coarse material. Water was added daily to make up for the loss by evaporation. The potassium dichromate was removed, before feeding, by dialyzing the cultures from 48 to 72 hours.

The cultures used in this experiment were obtained from various cases of coccidiosis. Culture A was obtained from the intestinal content of a yearling bull which was necropsied as a result of an acute coccidial infection. From microscopic examination, the culture appeared to contain about 90 per cent *Eimeria zurnii* and 10 per cent *Eimeria smithi*. Culture B came from an experimental case of coccidiosis in an Iowa calf. Culture C was obtained in Iowa and contained only *Eimeria smithi*. Culture D was sent to the laboratory for examination from Appomattox, Virginia. Microscopic examination showed it to be a very rich culture in which all of the oöcysts conformed to the morphologic description of *Eimeria zurnii*. Culture E was collected from six calves in Grayson County, Virginia, suffering from acute attacks of bovine coccidiosis. This culture later was shown to contain *Eimeria zurnii*, *Eimeria cylindrica* and *Eimeria smithi*. Culture F, containing oöcysts of *Eimeria zurnii* predominantly, was from a case of coccidiosis in Blacksburg, Virginia. Culture G came from a field case of coccidiosis in Iowa, and was obtained from the rectum of a cow with an acute attack of bloody diarrhea.

Source of the Infection

The histories of some of the field cases observed during this investigation have suggested certain possibilities which have had a marked influence on the course of this experiment.

In November, 1931, a 1,000-pound yearling bull, which died as a result of an acute coccidial infection, was brought to the laboratory for a postmortem examination. Death had resulted about one week after the onset of the first symptom and before marked emaciation had resulted. The lesions presented were typical of a severe, acute infection. Diffuse hemorrhages covered the entire surfaces of the rectum, colon, cecum and posterior part of the ileum. Areas throughout the colon and rectum were

entirely denuded of surface epithelium. The posterior portion of the intestines contained hemorrhagic fluid, shreds of epithelial tissue, and large organized blood clots with a diameter of about two inches. Oöcysts were found in abundance and a very rich culture (A) was obtained from the intestinal content. The diagnosis was confirmed further by a histologic examination. This was the first case of bovine coccidiosis observed by the owner, but he was positive that no animal on his farm ever had shown symptoms of the disease before. All the animals on the farm were old; there had been no additions made to the herd for several years, and the bull seldom came in contact with other animals. Therefore, the source of the infection could not be explained.

Several writers have pointed out that bovine coccidiosis is prevalent in certain districts in which the source of the infection cannot be determined. There is a possibility that immune animals or carriers act as spreaders of the infection. In a discussion of coccidiosis, the term "carrier" is used to designate only those animals which eliminate oöcysts without showing clinical symptoms of the disease. Robertson[4] believes that carriers or tolerants are the main sources of the infective organisms. Roderick[5] was unable to find oöcysts in the feces of cattle, either in healthy or affected herds, which did not present clinical symptoms of coccidiosis. Becker and Frye,[6] and other workers, have reported finding oöcysts in apparently healthy animals. It has been observed that apparently healthy cattle, in both infected and non-infected herds, occasionally may pass such small numbers of oöcysts that the organism may pass unnoticed in routine fecal examination. If the carrier, or tolerant, is responsible for the spread of the infection, it is at once apparent that it is impossible for the susceptible host to ingest a large infective dose at any one time. This assumption may be reached reasonably, without taking into consideration the very low percentage of sporulation attained by the organism under natural conditions. The evidence in the literature seems to support the theory that the infection is spread by tolerant or immune carriers. Otherwise, the unfavorable conditions encountered in nature soon would destroy the organism and the disease would disappear. Wilson[1] has shown that sunlight, drying, putrefaction, and winter temperature as encountered in Iowa, destroyed bovine coccidia.

The assertion that the natural infective dose of the organism is very small is supported further by the observation of two

cases of acute coccidiosis in month-old calves in a herd that came under our observation. Three calves were confined in a 10′ x 12′ concrete stall, bedded with straw. Grain which had been mixed in the feed-room was fed in troughs outside the stanchions, and milk was given from individual pails. The stalls were cleaned once a week, the troughs cleaned before each feeding, and the pails were sterilized with flowing steam between feedings. These calves never had been out of the stall. One died as a result of acute hemorrhagic enteritis, four days after the onset of the diarrhea. The remaining two calves then were moved and the stall was cleaned thoroughly.

Three young calves were placed in the same stall a month later. On February 4, 1932, one of these calves developed a diarrhea with an odor characteristic of intestinal hemorrhage. The next day the animal began passing shreds of epithelial tissue and a mixture of blood and mucus. The third day after the onset of the first clinical symptom, hemorrhage was profuse and the animal was killed for postmortem examination. Hemorrhagic areas were found throughout the rectum, colon, cecum, ileum and posterior part of the jejunum. Histologic examination showed extensive lesions in the rectum, colon, cecum and ileo-cecal valve. There were occasional lesions in the terminal portion of the ileum. A pure culture of *E. zurnii*, known as culture F, was collected from the intestinal content.

In attempting to discover the source of the infection, routine fecal examinations were run on all the calves in the barn, and litter from all the stalls was examined thoroughly for oöcysts. All but the two calves confined in the infected stall were negative for coccidia on this and subsequent examinations. These two animals were passing occasional oöcysts in which the cytoplasm had shrunk, and it was concluded that these oöcysts were picked up from the bedding. The two calves were moved to new quarters and the discharge of oöcysts stopped almost immediately. No clinical symptoms developed later.

Straw and litter, scraped from the floors of each stall, were examined, but no coccidia were found except from the stall where the infection had occurred.

Two non-infected, susceptible calves, three weeks old, were placed in the infected stall in an attempt to reproduce natural infection. The grain and hay were put on the floor in various places in the stall, making it possible for the calves to pick up a larger number of oöcysts than they would have if the feed had been put in the trough. It was hoped that natural infection

would occur and that oöcyst production and the resulting pathogenicity could be studied. The calves remained in this stall for three weeks and daily fecal examinations were made. From the second day there was a continuous passage of small numbers of degenerated and stained oöcysts of *E. zurnii* in the fecal discharge. A weekly examination of the litter in the stall was made, but only non-sporulated oöcysts could be found. After a period of three weeks, the animals were moved from this stall to the laboratory. The fecal examinations became negative for coccidia five days later, and remained negative until the animals were put on pasture.

The manner of feeding and the concentration of the oöcysts provided optimum conditions for infection, but it is apparent that no infective forms of the organism developed. On range or pasture there would be even less favorable conditions for infection. It seems apparent that natural infection is brought about by a more or less continuous ingestion of small numbers of coccidia, of which a very small percentage of oöcysts necessarily must be sporulated.

Pathogenesis Following Artificial Infection

Natural Resistance

A number of workers have had difficulty in reproducing coccidial infections experimentally. Roderick[5] suggests that some accessory or predisposing factors are involved in addition to the ingestion of ripened oöcysts. Tyzzer,[2] Fantham,[7] and others have noted that certain breeds of chickens seem to be more resistant to coccidial infection than others. There is no evidence in the literature studied which suggests a similar natural immunity in different breeds of cattle. In this experiment, in which more Holsteins than Jerseys were used, Jersey calves seemed to be more susceptible to artificial infection than Holstein calves, but there were exceptions in both breeds. The Holsteins adapted themselves to their environment and grew faster than the Jerseys. The variations noted probably resulted from individual resistance rather than breed immunity. Two calves appeared to possess natural immunity.

Calf 1, when it was two weeks old, was drenched with 500 cc of subculture A. Ten days later, the calf developed a severe diarrhea, which ceased four days later. No oöcysts were found in the fecal discharge until the 20th day. A careful examination of the slide from the centrifuged sample revealed only two oöcysts of *E. zurnii*. Fecal samples continued to be negative for oöcysts

for a period of 24 days following, at which time the examinations were discontinued.

Calf 2 was given 1,000 cc of mixed subcultures of culture A by means of a stomach-tube. Degenerated and stained oöcysts were passed on the fourth and fifth days. Fecal examinations were negative from the time of feeding until 34 days later. The calf then was drenched with 1,400 cc of subculture of culture B, a very rich culture in which *E. zurnii* predominated. On the seventh day, a severe diarrhea developed, and on the following day, the fecal discharge consisted mainly of mucus of an offensive odor. Only occasional oöcysts were passed on the 19th and 20th days, but on the 21st day the average count of oöcysts per low-power field was six *E. zurnii* and two *E. smithi*. The fecal examinations were negative until the 33rd day, when the calf passed a small number of oöcysts of *E. smithi*.

The results obtained with these two calves demonstrate a natural resistance. By the passage of a limited number of oöcysts, the animals showed a slight susceptibility, but the slight infection produced was not in proportion to the massive doses of infective material fed. There was no possibility of a tolerance having been developed, because the fecal discharges of the calves had been negative for coccidia from the time of birth.

Acquired Immunity

Coccidial infection in calves apparently confers an immunity of varying degree against subsequent infections. The type or duration of immunity conferred by one infection is unknown. Robertson[4] believes that the rareness of infection in older animals is a result of tolerance, or an immunity developed from infection early in life. Tyzzer,[2] and others, found that a well-marked and prompt immunity was established in the chicken by coccidiosis, especially from the species which penetrated deeply into the tissues. A well-marked immunity resulted in two calves from severe infections. In one of these cases the immunity was known to have endured for at least five months.

Calf 3, when three months old, was fed 1,200 cc of culture B. Twelve days later, there was a sudden onset of a fetid diarrhea, streaked with mucus and blood, and oöcysts appeared in great numbers. The discharge of oöcysts continued from the 12th to the 23rd day. About 95 per cent of the total oöcysts were *E. zurnii* and the remaining 5 per cent were *E. smithi*. The number of oöcysts passed gradually dwindled and the examinations were negative from the 31st to the 41st day.

Four days later, a second dose of 200 cc of culture A was administered. The calf remained negative on fecal examinations, and one month later the third dose, consisting of 300 cc of culture A, was fed. Fecal examinations were made daily for a month and a half, but no oöcysts were found. Five months after the primary dose was given, the calf was fed 1500 cc of an exceedingly rich subculture of culture B. No infection resulted, as was shown by repeated microscopic examinations of the fecal discharge.

Calf 4 was fed 1,000 cc of a mixture of cultures A and B. The passage of oöcysts started on the seventh day and continued until the 13th day. During this period there was a diarrhea of a watery consistency except on the ninth and tenth days. Then there was profuse hemorrhage with a passage of mucus and long shreds of epithelial tissue. The average count during the peak of the oöcyst production was 30 oöcysts of *E. zurnii* per high-power field. The second crop of oöcysts was passed from the 18th to the 32nd day, continuing over a much longer period than the first, but the oöcysts were not so numerous. They were predominantly *E. zurnii*, accompanied occasionally by small numbers of *E. smithi*. The calf had a diarrhea throughout the second period, but there was no perceptible hemorrhage. With a few exceptions the appetite remained fair to good. Fecal examinations remained negative after the 33rd day.

Two months after feeding the first infective dose, the calf was dosed with 1,000 cc of culture material collected during the first passage of oöcysts.

Primary infection in the two cases discussed apparently protected the calves against the ensuing infective doses given.

Species immunity is considered to be specific in calves as it is in chickens. Wilson[1] was able to produce species immunity in calves and could isolate pure cultures of coccidia by specific immunization. An attempt was made to establish in two calves a specific immunity for *E. smithi*.

Calf 5 was drenched with 400 cc of culture C, which was known to be a pure but very lean culture of *E. smithi*, in which only five or six oöcysts were found from an examination of a centrifuged sample. On the ninth day, a diarrhea with a characteristic odor of intestinal hemorrhage developed and the next day two oöcysts were found in a centrifuged sample. On the 17th day, the diarrhea again became marked and oöcysts were passed for four days, the maximum count averaging 20 per low-power field. Fecal

examinations were negative for a week. Oöcyst production started again on the thirty-first day and lasted eight days. An average oöcyst count showed the maximum number passed at any one time to be 20. On the 53rd day, 1,200 cc of culture D, an exceedingly rich culture of *E. zurnii*, was given in a drench. Two oöcysts of this species were found in a centrifuged sample on the tenth day. A slight diarrhea was present at this time but no infection resulted. This calf was drenched on the 72nd day of the experiment with 800 cc of culture E (mixed). Occasional oöcysts of *E. smithi* were passed but no infection resulted. A month later, the experiment was discontinued.

Calf 6 was drenched with 600 cc of a subculture of culture E, which had been sporulated in 2 per cent copper sulfate. A diarrhea streaked with hemorrhage developed on the 11th day, followed by oöcysts production on the 12th day. For a period of 13 days, the oöcysts of *E. smithi* were found in great numbers. Fecal examinations remained negative and, a month later, 200 cc of culture E was given. The calf had a foul-smelling, watery diarrhea until this time. Although occasional oöcysts of *E. zurnii* and *E. smithi* were found in the feces, no marked infection developed.

The results of these experiments would indicate a cross-immunity rather than a species immunity. As previous studies indicate, immunity is a local or cellular reaction rather than a serological reaction. It seems probable, therefore, that infection of calves with one species of coccidia would protect the animals against infection with another species, provided the site of the infections were the same. A variation in the site of the infection might explain the reported success of species immunization in calves. A mild infection with one species does not always protect the animal against subsequent infections with the same species. In the preceding experiments a heavy infection with *E. smithi* failed to provide complete protection against a mild infection later with the same organism.

The resistance and tolerance of an animal probably increases with its age. Active immunity probably is maintained during the life of the animal by the continued ingestion of small numbers of coccidia. The possibility of overcoming the resistance in an old animal by feeding a massive dose of infective material was tested in one cow.

Cow 1, an 11-year-old animal, was drenched with 2,000 cc of culture E. The animal was negative for Eimeria oöcysts when

the culture was administered. Stained and degenerated oöcysts were passed on the fourth day. From the sixth to the 11th day, large numbers of epithelial cells were found on microscopic examination of the feces. No infection developed, and the examinations were discontinued a month later.

Immunity in this animal probably was conferred by a mild infection during early life.

Other Factors

Dosage: The number of infective oöcysts fed to a susceptible calf does not determine the degree of the infection produced. Fish[8] in his work with Eimeria of the chicken, believes that there is no correlation between the size of the infective dose and the height and duration of the patent period. The most severe infections were obtained in two cases by feeding very small numbers of coccidia, while the response to massive doses varied from a mild to a well-marked infection.

Attenuation of oöcysts: In the experimental infection of 19 calves, there were no resulting mortalities. The cases of artificial infection did not assume the seriousness of any of the cases of natural infection observed. The difficulty experienced in producing a well-marked infection by feeding massive infective doses suggested the possibility of some necessary predisposing factors. Although it would be impossible in experimentally induced laboratory infections to reduplicate all of the factors encountered in natural coccidial infections, it might be possible to reproduce some of them. The most obvious difference between artificial and natural infections is the manner in which the oöcyst is sporulated. The question suggested itself as to whether sporulation of the oöcyst in an oxidizing reagent, such as potassium dichromate, might attenuate the parasite to some extent. An attempt was made to reproduce conditions for natural sporulation by placing the oöcysts in a soil culture. Two quarts of culture E were screened three times to remove organic material and added to an equal amount of sterilized soil. This was placed in a large, flat-bottomed porcelain pan, and enough distilled water was added to keep the culture moist at all times. The culture was aerated daily by a thorough mixing. No sporulated oöcysts could be found until a month later. A smear made from a centrifuged sample of this material revealed only one or two sporulated organisms, although there was an abundance of non-sporulated ones. Further development failed to take place and it was decided to test the viability of the segmented oöcysts and their

ability to produce infection by feeding a large quantity of this soil culture to a susceptible calf.

A quart of soil culture was repeatedly screened to removed the coarse particles of dirt. This material was given to calf 7 in small quantities and washed down with water. A mild diarrhea began on the ninth day and continued until the 23rd day, when it became severe. Straining was marked and numerous attempts were made to defecate. There was a hemorrhagic diarrhea with a foul odor. Marked symptoms of the disease were shown by the calf throughout the course of this experiment. Emaciation of the animal was noted on the 16th day, and it became so marked, eight days later, that the outline of the skeleton was visible. The animal was so weak during this period that it was unable to stand and was not expected to live. The eyes became sunken, the coat was dull, and there was a continual grinding of the teeth. There was no evidence of a secondary infection, for the temperature remained normal and the animal retained some desire to eat. A nephritis was manifested by the passage of bloody urine from the 18th to the 20th day. The passage of oöcysts continued over a long period. Small numbers of *E. smithi* were passed in pure culture from the ninth to the 17th day. During the ensuing ten days, large numbers of *E. zurnii*, *E. smithi*, and *E. cylindrica* were passed. After this period the fecal passages became normal and the animal made a rapid recovery in weight. Another period of diarrhea began on the 50th day and a small number of *E. smithi* were passed for five days. On the 60th day, oöcysts of an unidentified species were passed in small numbers. The average measurements for seven oöcysts were 42.6μ by 25.2μ. The feces then became negative for oöcysts and the experiment was discontinued. This calf demonstrates an instance of a typical parasitological condition. Although no definite conclusions can be drawn, this experiment indicates a possible difference between the virulence of oöcysts sporulating under natural conditions and those sporulated by artificial methods. If future experiments verify this theory, it should be of considerable economic importance in preventing losses from coccidiosis. An immunity might be developed in calves by feeding small amounts of oöcysts sporulated in potassium dichromate.

With this in mind, it was decided to test the effects of potassium dichromate cultures on farm calves. Five calves were dosed with 500 cc of culture E. Culture E had been collected from six calves on the same farm in a previous outbreak of coccidiosis.

This was the first outbreak of the disease. The healthy and infected calves always had been in separate pastures. These five calves were negative for Eimeria oöcysts before they were dosed with infective material. The owner was instructed to watch the calves carefully for symptoms. If the slightest diarrhea was manifested, he was to send fecal samples to the laboratory. No symptoms of the disease developed, the calves having remained healthy for five months. Fecal samples collected from the calves showed a mild infection with *E. smithi*, and also much larger oöcysts. The oöcysts of *E. smithi* measured 29.8μ by 19.8μ, while the larger oöcysts measured 46.8μ by 21.6μ.

Culture E, fed to these calves, did not produce marked infection, but it did produce a mild one which might result in protective immunity. No definite conclusions could be drawn from this experiment, but the suggested possibilities were that there was either an active immunity in these calves, or that the oöcysts fed had been attenuated to some extent by sporulating them in potassium dichromate. To determine just what does occur it will be necessary to dose a large number of calves shortly after birth and before an active immunity can be developed.

Self-limitation of the parasite: Tyzzer[2] concludes that coccidiosis in gallinaceous birds is a self-limited infection; that its duration in the absence of infection depends primarily upon its cycle of development, rather than upon the protective reaction of the host. in a recent article[9] he calls attention to the occurrence of great numbers of merozoites in the intestinal discharge, which is unfavorable to the coccidium in that its chances of propagation are diminished. Wilson[1] believes that possibly resistance or immunity is a greater limiting factor to the disease than the fact that there may be a limited number of asexual generations of the parasite. Clinical study of the Eimeria infections in calves, based upon the elimination of the oöcysts, indicates that the resistance of the animal has a marked influence in determining whether development of the merozoites will be sexual or asexual. Histologic study has shown that schizonts with daughter nuclei are developed within four days.

Calf 8 was fed 400 cc of culture E and killed for a postmortem examination; 196 hours later, macroscopic examination showed small patches of petechial hemorrhages throughout the rectum, colon and cecum. Centrifuged samples of the intestinal content from the different parts were negative for Eimeria oöcysts, with one exception. A smear made from a centrifuged sample of cecal

material contained two oöcysts of *E. zurnii* and three of *E. smithi*. These oöcysts showed a marked contraction of the cytoplasm, and they were believed to have been retained by the calf from the original infective dose. No sporozoites or merozoites could be found. Histologic sections showed only schizont formation with daughter nuclei.

It is probable that the first crop of merozoites is liberated about the fifth day, although none have been found in the fecal discharges. In a great many of the experiment calves, a marked diarrhea has been noted on the fifth day or soon afterward. It would be possible, as Tyzzer suggests, for a large number of merozoites to pass out in the feces. This would greatly decrease the number of oöcysts resulting from the infection. Merozoites in the colon and rectum would tend to be lost in this way, and those retained in the cecum would be responsible for the continued infection. This might explain the lack of noticeable hemorrhage in mild infections. If the animal's resistance was marked, the majority of the organisms might become sexually differentiated after the first asexual cycle, and great numbers of oöcysts would appear in the feces between the seventh and tenth days. In other cases a greater number of organisms continue asexual development, and only a few oöcysts are eliminated, until active immunity brings about an elimination of the parasites by influencing either a gradual or marked sexual development. The results obtained in this experiment do not indicate a cyclical character of development with a limited number of asexual generations for any of the species of Eimeria affecting cattle, but do tend to demonstrate that the influence of resistance or immunity of the host is responsible for the apparent self-limitation of the parasite.

RESULTS

Effects of Temperature

The minimum temperature at which bovine coccidia are heat-killed was established by Wilson.[9] His results closely agree with those of other investigators. Other questions concerning the effects of a minimum temperature have not been answered.

Two hundred cc of culture E were placed in an electric refrigerator at $-5°C$. The culture remained frozen from January 5, 1932, until March 3. Microscopic examination showed that there had been a slight decrease in the number of oöcysts, proved by finding the shells of the degenerated oöcysts. An equal amount of 1.5 per cent potassium dichromate was added to the culture

when it was placed at room temperature. The culture was aerated daily for 11 days, at which time an examination showed that 95 per cent of the oöcysts had sporulated. The culture was returned to −5°C. on March 15. It was taken from the refrigerator on April 30, and examined. No oöcysts could be found. This culture was five months old at the termination of this experiment and the time element probably played a part in the destruction of the oöcysts.

A portion of culture E was sporulated, washed free from the potassium dichromate, and exposed to outdoor winter temperature for five days. The culture then was allowed to melt at room temperature and was examined. There was no perceptible destruction of the oöcysts. It was placed at −5°C. on March 15, and removed April 30. On examination it was found that about 50 per cent of the oöcysts had been broken. Exposure at a low temperature in this case was not so long as in the preceding experiment. This culture also was free from potassium dichromate.

A sporulated sample of culture E was exposed to winter temperature from January 5 to April 30, 1932. A pint fruit jar was used as a container. The preservative had been removed from the culture by dialization for 72 hours. Examination on May 1 showed that there was an approximate 50 per cent decrease in the number of oöcysts.

A non-sporulated portion of culture E was exposed under the same conditions and for the same period. It was estimated that about 25 per cent of the total number of oöcysts had been destroyed by the exposure. An equal amount of 1.5 per cent potassium dichromate was added and the culture was aerated daily for ten days. About 80 per cent of the oöcysts segmented during that time. It would seem that under favorable conditions bovine coccidia would not be killed by exposure to Virginia winter temperatures. Non-sporulated oöcysts are possibly more resistant to low temperatures than are the segmented forms. The minimum temperature necessary to destroy oöcysts by a limited exposure has not been determined.

Age and Viability

No evidence can be found which demonstrates how long bovine coccidia will endure and retain their infective properties. Most investigators probably consider the average life of their cultures to be about five months. It has been noted, during the course of this experiment, that most of the sporulated oöcysts disappear from a culture in six or seven months. The following ex-

periment shows that *E. smithi* retains its power of sporulation and infectiveness for 18 months.

Culture C, a pure strain of *E. smithi*, was collected from an infected calf in Iowa, on May 26, 1930. It was preserved in 2 per cent potassium dichromate and aerated daily until sporulation had resulted. It was stored at 22°C, until June 10. When the culture was examined June 25, 1931, no Eimeria oöcysts could be observed microscopically, although what appeared to be oöcyst shells could be seen. A large amount of this culture was dialyzed and fed to two calves. A three-month-old calf received 1,000 cc and the other calf, eight days old, was given 300 cc. No infection resulted in either animal. The remainder of culture C was returned to storage. When it was examined again on October 8, it contained ice from having been solidly frozen in the electric refrigerator. Microscopic examination revealed the fact that a very small number of non-sporulated oöcysts of *E. smithi* previously had been overlooked. These oöcysts had been considered as infertile when the original culture had been sporulated. Fresh preservative was added and the organisms were aerated three or four times daily. Sporulation was completed by October 28, and 400 cc of this material, which contained only about five oöcysts in a centrifuged sample, was given to a susceptible calf on November 14. A marked infection resulted and a pure culture of *E. smithi* was recovered. (Refer to discussion on calf 5.)

The culture utilized in this experiment contained both sporulated and non-sporulated oöcysts when it was stored first, but the sporulated oöcysts had disappeared after a year. It was shown clearly that the material had lost its infective power after the destruction of the segmented oöcysts. It is apparent from this experiment that non-sporulated oöcysts are more resistant than the sporulated form of the organism.

Effects of Disinfectants

Two cultures (A and E), which contained oöcysts of *E. zurnii*, *E. smithi* and *E. cylindrica*, were used for this experiment. The freshly collected fecal material was screened three times through wire mesh to break up large particles and remove organic material. After the oöcysts were exposed to the action of the disinfectants, as indicated in table I, they were removed by washing four times in distilled water and centrifuging. The cultures were incubated in 1.5 per cent potassium dichromate. Large test-tubes, about three-fourths full of culture, and tightly corked, were used as containers. The corks were removed for a short

time; the tubes were aerated twice a day by shaking thoroughly, and the tubes then were placed in a horizontal position to give a maximum amount of surface. The tests were conducted for periods of three weeks, which allowed ample time for sporulation. The percentage of sporulation was based on a count of 100 oöcysts from centrifuged samples. Table I represents the results of two trials.

The repeated centrifuging had no effect, because 95 per cent sporulation resulted in the control tubes.

No development took place when the cultures were incubated in 4 per cent formaldehyde, or 2 per cent liquor cresolis compositus, but 95 per cent of the oöcysts developed in 2 per cent copper sulfate.

TABLE I—*Effect of disinfectants.*

TIME OF EXPOSURE	48 HOURS			1 HOUR			10 MINUTES		
Phenol (%)	0.5	2.5	5.0	1.0	2.0	5.0	2.5	5.0	10.0
Sporulated (%)	0	0	0	72.0	0	0	0	0	0
Mercuric chlorid (%)	0.1	0.2	0.3	0.1	0.2	0.3	0.3	0.5	1.0
Sporulated (%)	0	0	0	0	0	0	0	0	0

Effects of Sunlight and Drying

Freshly collected cultures of bovine coccidia were poured in open Petri dishes and exposed to the direct rays of the sun for a total of 24 hours. No preservative was added to two of the dishes, while the other two contained 1.5 per cent potassium dichromate. Preservative was added to the cultures and they were placed in test-tubes and aerated daily for three weeks, but no development took place. The same results were obtained from a later trial.

Two Petri dishes of culture, one of which contained a preservative, were dried for 15 days at 20°C. Two other identical samples were dried for 15 days out of doors, exposed to sunlight and a mild temperature. Water was added to dissolve the dried material. Only oöcyst shells were found upon the examination of centrifuged samples. A portion of culture E was added to sterilized soil and allowed to dry for 20 days. Examinations at intervals showed decreases in the number of oöcysts. Only oöcyst shells could be found at the end of the period.

A subculture was placed in a 12″ x 20″ flat-bottomed porcelain

pan. The depth of the culture was about one-eighth of an inch. This was dried at 20°C. for 48 hours and examined. Nothing but oöcyst shells remained. This test was repeated with similar results.

Fecal material, of a solid consistency, containing large numbers of oöcysts, was allowed to dry for 15 days at room temperature. No oöcysts could be found after this period of drying.

Effects of Putrefaction

Freshly collected samples of bovine coccidia containing a large amount of hemorrhage were placed in the incubator and paraffin-oven. The temperature of the incubator was 37°C., while the temperature in the paraffin-oven was about 58°C. The cultures were removed after a month of exposure to these temperatures and 1.5 per cent potassium dichromate was added. They were aerated daily for a period of three weeks, and then examined. No sporulation had taken place; the oöcysts still retained their shape, but they showed a marked contraction of the cytoplasm.

Fresh material was placed at −5°C. for one month. Preservative was added and the culture was aerated daily for two weeks. Ninety-five per cent of the oöcysts segmented. The hemorrhage in this sample caused no destruction of the oöcysts at this low temperature.

A pure culture (D) of *E. zurnii* containing almost pure hemorrhage stood at room temperature for five days. Preservative was added and the culture was aerated daily for a week. Complete sporulation resulted and there apparently was no marked destruction of oöcysts.

Screened cultures of oöcysts in which no visible hemorrhage was present have stood at room temperature, with no preservative, without marked destruction of oöcysts. The viability was shown by sporulation trials. A large amount of the same culture was stored at 22°C. for two months. Rapid sporulation of the oöcysts took place when 2 per cent copper sulfate was added and the culture aerated.

Bovine coccidia are not so susceptible to putrefactive changes as is generally believed. Non-sporulated oöcysts probably are more resistant to these changes than the segmented forms. It would seem that putrefactive changes would be more severe if intestinal hemorrhage were present in the feces. Oöcysts present in manure piles probably are readily destroyed by decomposition and the resulting high temperature.

Artificial Digestion

Artificial digestion trials were made with 12 hooded rats confined in the laboratory. Two rats were killed. The intestines were stripped of fecal material, and centrifuge samples were run. No coccidial oöcysts were found. The remaining rats were starved for two days, then a very rich portion of culture E was fed in milk. Two rats were killed one hour later, four more in 24 hours, and the remaining four were killed 48 hours after feeding. Sporulated and non-sporulated oöcysts of bovine origin were found in the intestinal content of all the rats. One of the last ones to be killed showed a marked hemorrhagic enteritis in the small intestine. An examination showed a rather heavy infection of coccidia. A few sporulated and non-sporulated oöcysts of *E. zurnii* and *E. smithi* were found. It was not difficult to distinguish between bovine coccidia and the coccidia of the rat, because the latter were quite large, and although they were somewhat ovoid in shape, there was a distinct point at the small pole.

To check this experiment further, it was decided to repeat the trial. Seven half-grown rats were placed in a sterile cage and fed sterilized grain and pasteurized milk. Composite fecal samples were negative for coccidia for two weeks. The rats were dosed three times with culture E fed in milk with a pipette. Both sporulated and non-sporulated oöcysts were passed in the feces. After the last feeding, two rats were killed in 1 hour, two in 24 hours, and two in 48 hours. Fecal examination showed undigested oöcysts in all cases. The finding of segmented oöcysts of bovine coccidia in the intestine of a rat after 48 hours would indicate that no digestion of the oöcysts had resulted.

Sporulation Trials

Methods: Potassium dichromate is used universally as a preservative because it retards putrefaction and furnishes some oxygen to the cultures. Very little is known of the factors necessary for natural sporulation.

A number of attempts were made to sporulate bovine coccidia in the absence of a preservative, and to reduplicate, as much as possible, the required factors of natural sporulation.

Two quarts of sterilized dirt and culture E were mixed thoroughly, and placed in a 12″ x 20″ flat-bottomed, porcelain pan. The depth of the culture was three-fourths of an inch. It was stirred daily to aerate it and just enough water was added to

keep the dirt saturated. The experiment continued for five weeks, but only an occasional sporulated oöcyst could be found in samples centrifuged repeatedly. No odor of putrefaction in this culture could be detected.

The same procedure was followed in preparing another soil culture. This material was spread one-eighth inch deep in a box 2 by 3 feet. A wet towel was spread over the culture daily to supply moisture. Although the oöcysts could be found in great numbers in centrifuged samples, no sporulated ones were seen.

Fecal material, containing enormous numbers of coccidia and an equal amount of sterilized soil, were placed in a half-gallon fruit-jar. Water was added to make up for evaporation. The culture was aerated once or twice daily by forcing air to the bottom of the solution with a pressure bulb. Only an occasional sporulated oöcyst could be found, but the material later was proved to be infective by feeding it to a susceptible calf. A small portion of this material was kept at 37°C. for three weeks, but no development of the organisms resulted.

A pail of litter was collected from an infected stall in the college dairy barns and kept in the laboratory. Water was added regularly to keep the material moist. Only one segmented oöcyst was found, although repeated examinations were made. Sporulation probably was prevented by the continued decomposition that occurred. No sporulation took place in cultures of bovine coccidia to which only water had been added.

Two per cent copper sulfate was tried as a preservative for cultures and compared with 1.5 per cent potassium dichromate. No difference could be noticed in the rate or percentage of sporulation.

Sporulation time: The sporulation periods for the different species of bovine coccidia reported by various investigators have varied. These variations probably result from failure to provide optimum conditions for sporulation. Tyzzer[2] pointed out that rapid and maximum sporulation of chicken coccidia can be attained by placing small amounts of the potassium dichromate culture in open Petri dishes exposed at room temperature. This is unsatisfactory to sporulate bovine coccidia when there is a large amount of fecal material. The sporulation times given by Wilson[1] are as follows: *E. cylindrica*, 2 to 10 days; *E. zurnii*, 12 to 20 days; *E. smithi*, 14 to 21 days.

Bovine coccidia, when aerated in large containers, do not become infective in a short period. Three quart fruit jars, each containing 200 cc of a subculture of culture B, were placed at

37°C. and aerated four or five times daily. This culture contained oöcysts of *E. cylindrica, E. zurnii* and *E. smithi.* Before the calves were dosed, the oöcysts were freed from the potassium dichromate by washing and centrifuging the material four times.

Calf 10 was drenched with 200 cc of subculture B, which had been incubated 24 hours. Fecal examinations were made for a month. No infection resulted.

Calf 11 was given 200 cc of subculture B, which had been incubated 72 hours. No infection was produced. This calf later was proved to be susceptible by feeding 150 cc of culture A.

Calf 12 received 200 cc of subculture B, which had been 120 hours in the incubator. Fecal examinations remained negative for Eimeria oöcysts.

The possibilities for rapid sporulation were suggested when centrifuged samples, which had stood for 12 hours, were examined and sporoblast formation was noted in oöcysts of *E. cylindrica* and *E. zurnii.* There was no apparent development in *E. smithi.* About 200 cc of a subculture of culture E, containing about 90 per cent *E. zurnii,* 7 per cent *E. cylindrica,* and 3 per cent *E. smithi,* was screened and placed in a 6″ x 8″ flat-bottomed pan. Potassium dichromate and a small amount of concentrated sugar solution were added. Sixty per cent of *E. cylindrica* sporulated after incubation for 24 hours, and appeared to be fully sporulated when examined after 48 hours. Sporoblast formation had taken place in *E. zurnii* in 24 hours and after 48 hours 35 per cent were fully segmented. Complete development had occurred at 72 hours. Sporoblast formation was noted in an occasional oöcyst of *E. smithi* after incubation for 72 hours. An examination after 96 hours showed that plasmolysis had caused a marked destruction of *E. zurnii* and that a few oöcysts of *E. cylindrica* had degenerated. About 1 per cent of *E. smithi* were sporulated. This material was dialyzed.

Calf 13 was drenched with this subculture of culture E. On the 12th to the 17th day, *E. zurnii* were passed in small numbers. Large numbers of *E. cylindrica* were passed in pure culture from the 23rd to the 28th day. On the day following, *E. cylindrica* and *E. smithi* were found. Oöcysts were passed in great numbers for a short time. A diarrhea developed on the 21st day and on the 26th day the feces were streaked with hemorrhage. No severe symptoms of the disease were shown.

Freshly collected feces, containing large numbers of *E. cylindrica,* were screened and potassium dichromate was added. This culture was added to a flat-bottomed pan to a depth of a quar-

ter of an inch. Complete sporulation had occurred in 95 per cent of the oöcysts in 48 hours. The same results occurred in two succeeding trials with the same organisms. Pure cultures of *E. smithi* were treated in the same manner, and sporulation occurred in 3 to 5 days. The sporulation time is given for the three species of Eimeria in cattle:

Eimeria cylindrica — 24 to 48 hours
Eimeria zurnii — 48 to 72 hours
Eimeria smithi — 3 to 5 days

To test the infectiveness of *E. cylindrica* after incubation for 48 hours, a pure culture of the organism was fed to a susceptible calf (14). Elimination of *E. cylindrica* in pure form began on the tenth day.

Morphologic and physiologic characteristics of the genus Eimeria: Observation were made on the morphologic and physiologic characteristics of the genus Eimeria affecting cattle. These are recorded in table II.

The following data were computed from the measurements of 50 consecutive oöcysts, as they appeared on a slide, from pure cultures of two species of coccidia:

*Eimeria cylindrica**

Major axis		Minor axis	
s d	$= \pm$ 1.23	s d	$= \pm$ .2
rs	$= \pm$ .9	rs	$= \pm$ .135
rm	$= \pm$ .2	rm	$= \pm$ .04

Eimeria smithi

Major axis		Minor axis	
s d	$= \pm$ 1.06	s d	$= \pm$ .279
rs	$= \pm$ .74	rs	$= \pm$ .6
rm	$= \pm$ .23	rm	$= \pm$ .2

Experimentation with pure cultures of bovine coccidia has shown that the morphologic and physiologic features of the subcultures are identical with those of the parent cultures. With careful consideration of the evidence, it becomes apparent that *E. zurnii, E. cylindrica* and *E. smithi* are three separate and distinct species of bovine coccidia.

Examination of culture E has shown the presence of very large oöcysts which measure about 46.2μ by 25.2μ. The oöcysts are so large that the elongated sporocysts float free inside. The sporocysts have a large residual body. The oöcyst wall is stained a dark green color. Many investigators have stated that the

*s d = standard deviation.
rm = mean.
rs = error (single observation).

oöcyst wall of *E. smithi* may be either colorless or stained. Only one of the many cultures used during this study contained these large stained oöcysts, and when this culture was fed to calves, considerable numbers of the oöcysts passed during the course of the infection. No stained oöcysts were found in any of the several pure cultures of *E. smithi*. The oöcysts are egg-shaped and they sporulate in about five days. From a careful comparison of these large oöcysts with those of *E. smithi*, it is believed that this large form of Eimeria is a distinct and unnamed species of bovine coccidia.

TABLE II—*Comparisons.*

CHARACTERISTICS	EIMERIA ZURNII	EIMERIA CYLINDRICA	EIMERIA SMITHI
Sporulation time	48 to 72 hours	24 to 48 hours	3 to 5 days
Major axis	18μ	23.7μ	28μ
Minor axis	17.3μ	14.4μ	20.5μ
Shape of oöcyst	Spherical	Cylindrical	Egg-shaped
Oöcyst wall	Uniform, colorless	Uniform, colorless	Thin at small pole, colorless
Residual body	None	In spore	In spore
Shape of sporocysts	Ovoid	Cylindrical	Elongated

SYMPTOMS

The symptoms of bovine coccidiosis have been well described in the literature. The severity of the symptoms shown in these experiments varied markedly. The predominant symptom is diarrhea, which is usually mild at the onset of the disease. This may change rapidly to a severe form with marked hemorrhage, when large amounts of mucus and long shreds of epithelium are passed with the feces. A nephritis, which is manifested by the passage of bloody urine, may accompany the diarrhea. If the resistance of the animal is very low, secondary intestinal infection, or pneumonia, may result. Emaciation is rapid. The animal eats, but the appetite is poor. The eyes are sunken, the haircoat rough, the pulse is accelerated, and a general anemic condition is apparent. The animal becomes dull and listless, and will stand with the back arched and the abdomen drawn up. Severe intestinal pain is evidenced further by a grinding of the teeth and a drooling of saliva. Calves recuperate very slowly from severe infections.

PATHOLOGY

The gross pathologic changes in bovine coccidiosis are confined to the rectum, colon and cecum. They are loss of surface epithelium, hemorrhage, and a thickened and cooked appearance of the mucosa. Large areas may be denuded of the epithelium entirely. The hemorrhages vary from petechial to diffuse. These lesions are noticed usually on the crests of the folds of the lining membrane. The three species of bovine coccidia invade the deeper portions of epithelium in the intestinal glands, where all developmental stages may be found. The rectum usually is affected more than the other portions of the intestine, although occasionally the lesions will be more numerous in the cecum. Catarrhal enteritis may be present in the small intestine, but microscopic lesions rarely are found there, except at the terminal portion of the ileum. No marked differences in the developmental stages of three species could be found, but the sexual form of *E. smithi* are much larger than the other two species.

Discussion

In experimental work with bovine coccidiosis, it is important to secure susceptible, non-immune calves. All possible sanitary precautions must be taken to prevent accidental infection. Accidental infection does not occur in cattle so easily as in other animals. This probably is due to a difference in the physiologic characteristics of the oöcysts. It is important to use pure cultures of the different species to avoid confusing results.

Results of experimental and field cases of coccidiosis indicate that natural infection occurs through the ingestion of small numbers of ripened oöcysts, possibly over a long period. The infection probably is spread in a herd by immune animals acting as carriers of the organisms. It seems quite evident that animals have a natural resistance and that a protective immunity against subsequent infections may result from a severe attack of coccidiosis. No fatalities occurred among the 19 calves artificially infected, and there were no correlations between the size of the doses given and the resulting infection. This suggests some predisposing factor which influences the course of the infection. There is slight evidence to show that oöcysts which are sporulated without a preservative produce a more severe infection than those sporulated in 1.5 per cent potassium dichromate. A study of the courses of the infections indicates that the resistance of the host has a direct influence on sexual or asexual development of the parasite.

Both sporulated and non-sporulated oöcysts have a low thermal death point and are destroyed by sunlight, drying and putrefaction. The oöcysts are very resistant to freezing temperatures. The number of ooöcysts in a culture decreases with age. Non-sporulated oöcysts may retain their ability to sporulate and their infective power for at least a year and a half.

Artificial digestion trials have shown that rats will not digest bovine coccidia. A large percentage of bovine coccidia will not sporulate unless some preservative is added to the culture. Sporulation trials have shown that great numbers of the oöcysts of three species of coccidia will sporulate and become infective in a very short time under favorable conditions. The different morphologic and physiologic characteristics of three species of coccidia have been noted. Pure cultures of these species retain their characteristics after repeated infections. It is concluded that *E. zurnii*, *E. smithi* and *E. cylindrica* are valid species.

Conclusions

1. Much of the past confusion concerning bovine coccidiosis has been eliminated by recent experimentation.

2. Natural infection probably results from a continuous ingestion of small numbers of ripened oöcysts, immune carriers spreading the organism.

3. The natural resistance of calves may vary. An acquired immunity, which protects the animal against subsequent infections for at least five months, may result from a severe coccidial infection. Old animals, as a rule, probably have a well-developed immunity.

4. In this study there was found to be no correlation between the amount of infective material fed to a susceptible calf and the resulting infection.

5. Failure to produce severe artificial infections has suggested the possibility of some predisposing factor. It is believed that sporulation of the oöcysts in potassium dichromate may attenuate them to some extent.

6. There does not seem to be a definite cyclical character of development with a limited number of asexual generations in bovine coccidiosis. Evidence indicates that the course of the infection is influenced by the resistance, or immunity, of the host, which determines whether the development of the parasite will be either sexual or asexual.

7. Non-sporulated oöcysts will probably survive the winter temperatures encountered in Virginia.

8. Non-sporulated oöcysts seem to retain their viability at refrigeration temperature longer than sporulated ones.

9. Both non-sporulated and sporulated oöcysts are destroyed readily by the action of sunlight, drying and putrefaction.

10. Sporulated oöcysts taken from cattle and fed to young rats were not digested.

11. It was found impossible to sporulate any great numbers of bovine coccidia unless a preservative was used. Copper sulfate seems to be as satisfactory as potassium dichromate as a preservative for sporulating bovine coccidia.

12. Sporulation time for some of the species of bovine coccidia is not so long as is commonly believed.

13. The morphologic characteristics of one type of oöcyst encountered appeared not to conform to any of the described bovine species.

REFERENCES

[1]Wilson, I. D.: A study of bovine coccidiosis. Va. Agr. Exp. Sta. Tech. Bul. 42 (1931), pp. 1-42.

[2]Tyzzer, E. E.: Coccidiosis in gallinaceous birds. Amer. Jour. Hyg., x (1929), pp. 269-383.

[3]Benbrook, E. A.: A demonstration on the diagnosis of internal parasites of domestic animals. Vet. Pract. Bul., ix (1929), pp. 5-24.

[4]Robertson, A.: Coccidiosis in calves. Vet. Jour., lxxxvii (1931), 7, pp. 312-325, and 8, pp. 351-385.

[5]Roderick, L. M.: The epizoölogy of bovine coccidiosis. Jour. A. V. M. A., lxxiii (1928), n. s. 26 (3), pp. 321-327.

[6]Becker, E. R., and Frye, . W.: *Eimeria ellipsoidalis Nov. spec.*, a new coccidium of cattle. Jour. Parasitol., xv (1929), pp. 175-177.

[7]Fantham, H. B.: The morphology and the life history of *Eimeria avium*. Proc. Zoöl. Soc., (1910), p. 672.

[8]Fish, F.: The effects of physical and chemical agents on the oöcysts of *Eimeria tenella*. Sci., lxxiii (1931), pp. 292-293.

[9]Tyzzer, E. E., Theiler, H., and Jones, E. E.: Coccidiosis in gallinaceous birds. II. A comparative study of the species of Eimeria of the chicken. Amer. Jour. Hyg., xv (1932), pp. 319-393.

Work for Your Profession

If you work in a profession, in heaven's name work for it. If you live by a profession, live for it. Help advance your coworker. Respect the great power that protects you, that surrounds you with the advantages of organization, and that makes it possible for you to achieve results. Speak well for it. Stand for it. Stand for its professional supremacy. If you must obstruct or decry those who strive to help, why—quit the profession. But as long as you are a part of a profession, do not belittle it. If you do, you are loosening the tendrils that hold you to it, and with the first high wind that comes along, you will be uprooted and blown away and probably you will never know why.

—CHARLES E. DAWES.

TRAINING POLICIES FOR VETERINARY RESERVE OFFICERS*

By J. G. HORNING, *Houston, Tex.*

The main object of the training policies for veterinary reserve officers is to prepare them for performing efficiently the duties of their mobilization assignments, and to prepare them for promotion to the next higher grade. The responsibility for this training rests with the corps area commanders who are charged with the supervision, coördination and control of Organized Reserve Corps and units of the Organized Reserve within their respective corps areas; subject to the supervision and coördination of the War Department.

The training policies for veterinary reserve officers, as established by the War Department, are splendid in every respect and so arranged with the individual's convenience in mind, that the progress made in pursuing training for credits depends entirely upon the time and work given to it. Whether the officer is on the inactive or active status determines the policy of the training he receives; the facilities for individual training on an inactive status are:

1. The Army extension courses.
2. Courses of instruction at the troop schools and group schools.
3. Attendance at contact camps.
4. Duty on various boards of officers on inactive status.
5. Firing practice on an inactive status with units of the Regular Army.
6. Inactive duty at special assemblies of officers for individual training in the tactics and technic of his arm or service.
7. Attachment for training at his own expense to the Regular Army or with proper consent of state officials with units of the National Guard, on an inactive status.
8. Duty on an inactive status at special service schools.

The above cover the necessary methods that can be used by the officer on an inactive status to prepare himself in the wartime duties of his grade and assignment and prepare himself for promotion to the next higher grade.

Easily the best course for credit, as well as personal improvement, is that covered in the Army Extension Courses. These correspondence school lessons are so arranged as to be worked

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out easily by the student and if any trouble is experienced the unit instructor is always more than willing to help.

Troop schools and group schools provide a chance for listening to occasional lectures on subjects of general interest to the service as well as personal instruction; as a means to brush up in various subjects, to receive aid for some correspondence problem and for pleasant contact with brother officers of the section in which one resides.

Contact camps are what they imply, usually arranged for a series of lectures and instructions in various subjects for the good of the service, with plenty of time for personal contacts with brother officers of the section in which one lives and meeting the unit instructors of the various branches of the service stationed at Reserve Headquarters. Duty on various boards of officers on inactive status provides pleasant contact and credits. Firing practice and attendance at special assemblies for individual training provide instruction with further contacts.

Attachment for training with the Regular Army or National Guard at his own expense provides instruction, contacts and a splendid vacation in pleasant surroundings, providing one has the time and money to do this. The best policy for individual training on active status, arranged according to my personal idea on the subject, is the following:

1. Utilization of the 14-day training period.
2. Special course at the Service School.
3. Active duty with the Regular Army or with the consent of the proper state officials with a unit of the National Guard.
4. Duty on active status on various boards of officers.
5. Duty at special assemblies of reserve officers for individual training in the technic or tactics of an arm or service.
6. Active duty as instructor at Citizens Military Training Camp.

Preference for the 14 days of active duty training will be given those officers who prepare themselves therefor by pursuing appropriate extension courses. A reserve officer when ordered to active duty will receive the mileage prescribed by law from his home to his station and from his station to his home. Pay status begins on the date when an officer complies with order calling him to active duty and ends on the date when he is actually relieved from active duty. Pay and allowances for the 14-day period are as follows:

Second Lieutenant	$ 85.40
First Lieutenant	122.58

Captain	147.48
Major	188.54
Lieutenant Colonel	217.31
Colonel	228.35

Reserve officers on active duty are entitled to longevity pay at the rate of five per cent of their base pay for each three years of service. As can be seen here, one has a pleasant two-week vacation, with pay, congenial companions and a decided change from his everyday life. There is ample opportunity to study and acquire much useful information from direct observation. The combination of the Army extension courses with the 14-day training period is the ideal method for creating an efficient Reserve Corps.

In the training with the unit or a unit of the Regular Army, in some cases it is done as a school with classes and lectures in the morning and early afternoon, a short course on the actual things necessary for every officer to know, this affording contact usually with one's own unit or attached to some unit and quartered at some Army Camp for instruction by Regular Army officers. This is pleasant but in my opinion not so good as training with the troops during manœuvres. A reserve regiment attached to a regular regiment of the same branch of the service affords the reserve officers an opportunity to study and observe the actual procedures that are necessary for an officer to perform in camp or in the field. The splendid coöperation of the regular army officers makes this ideal for training. This in conjunction with home Army extension course study, and contact camps is the best system for training officers without any previous experience and also to afford opportunity for the old campaigner to keep up to the minute in his Army work. It all resolves on the individual's actual desire to excel in his work.

Where one has the time and can show sufficient merit, there is a possibility for selection to attend the special service school. This is very desirable but attained by very few officers of the Veterinary Reserve.

Active duty with the Regular Army comes under the same classification as utilization of the 14-day period in regard to training and credit. Duty on various boards and special assemblies is covered with the same credits as inactive duty. In struction at Citizens Military Training Camps is usually carried out by officers of the other branches of the service and, when carried out by Veterinary Reserves, would be given the same credits for hours on duty with pay according to rank.

The system arranged can hardly be improved upon. The time

and enthusiasm of the individual govern entirely the progress he will make. When called on for lectures before the Reserve officers it would be well to mimeograph your lecture for the further home study of the individual, as even in taking notes one is liable to miss certain parts, especially in any technical subject. Literature for study by the student can be had through the Reserve headquarters in his community. Application for the *Army Veterinary Bulletin*, published at the Field Service School, Carlisle Barracks, Pennsylvania, enrollment in the Reserve Officers Association of his community, and subscribing to the veterinary journals of this country are essentials necessary to being proficient veterinarians as well as reserve officers.

Best Dog Poem

Dog World, published at 3323 Michigan Blvd., Chicago, Ill., has established the Dog World Annual Award for the best dog poem. The first award will be for the year 1933. Manuscripts must be received by mail, on or before December 31, 1933.

There is no limitation on the length of the poem or form of verse. It can be serious, humorous, long or short, lyrical, inspirational, or just doggerel. Manuscript will not be returned unless stamped and addressed envelope is furnished.

There will be 10 cash prizes: first prize, $25; second prize, $10; third prize, $5, and seven additional prizes of $1 each, for the ten best poems respectively. The award will be made annually for each calendar year and on the conditions set forth.

The awards for 1933 will be announced in the February, 1934, issue of *Dog World*. The judges for 1933 are Richard Finnegan, Editor of the *Chicago Daily Times;* Dr. H. Preston Hoskins, Secretary-Editor of the American Veterinary Medical Association, and Capt. Will Judy, Editor of *Dog World*.

Bausch & Lomb Exhibit at World's Fair

During the Century of Progress Exposition at Chicago, the Bausch & Lomb Optical Co., of Rochester, N. Y., will have an exhibit of instruments in the Hall of Science (spaces 1 and 2). Apparatus will be demonstrated upon request. Veterinarians are invited to visit the exhibit.

Come to Chicago—August 14-15-16-17-18

ANAPLASMOSIS

III. A Further Experimental Demonstration of Its Identity in Louisiana*

By G. DIKMANS, *Washington, D. C.*
Zoölogical Division, Bureau of Animal Industry
U. S. Department of Agriculture

Having made a tentative diagnosis of anaplasmosis on the basis of our first preliminary experiments, in Baton Rouge, La., the work was now transferred to our official station at Jeanerette, La., where we were provided with an experiment barn through the coöperation of the Division of Animal Husbandry. It was considered necessary to compare our disease in various respects with anaplasmosis as reported from other parts of the world. The reasons for this procedure were as follows:

Anaplasmosis, although unrecognized as a distinct disease entity, was observed by Smith and Kilborne as early as 1893. These authors considered it as a mild form of Texas fever, transmitted by the fever tick, *Boöphilus annulatus*. The tick eradication campaign by means of periodical dipping was inaugurated in 1906, and even after Theiler had demonstrated, in 1910, that anaplasmosis was a disease distinct from piroplasmosis or tick fever, it was still rather tacitly assumed, without discussion or even much consideration, that since both diseases were carried and transmitted by the cattle fever tick, *Boöphilus annulatus*, the destruction of this tick would result in the eradication of both of these diseases. For almost twenty years it appeared that this opinion was correct. During the period of 1906 to 1924, large areas were released from quarantine and declared tick-free, *i. e.*, free from the cattle fever tick, *Boöphilus annulatus*, and from the areas so released there were no reports of tick fever or of diseases resembling tick fever.

About 1924, reports began to come in about the occurrence in tick-free areas of Louisiana of a serious disease of cattle, resembling tick fever in its general clinical manifestations, except for the absence of hemoglobinuria or red water and with an intense yellow discoloration of the visible mucous membranes followed by the dead or porcelain-white appearance of severe anemia. At no time were ticks reported as being found on the sick animals. This disease received the common names of "yellow jaundice,"

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"tickless tick fever," etc. These reports were shortly followed by similar reports from other tick-free area and even from areas which had never been included in the old original tick-quarantine area. Although by now diagnosed as anaplasmosis, this diagnosis rested upon clinical observations rather than on experimental evidence, and the questions naturally arose: Is this disease really anaplasmosis, and, if it is, what particular carrier is now responsible for its spread in the absence of the cattle fever tick? The importance of determining these questions beyond cavil is self-evident.

Anaplasmosis as a disease has certain characteristics by which it may be known. These characteristics are:

(a) The clinical symptoms; (b) the postmortem pathology; (c) the appearance of the "marginal points" in stained blood-films; (d) the alterations in the blood; (e) the apparent impossibility of increasing the virulence by successive passage through susceptible animals; (f) the ability of Anaplasma to pass through and persist in sheep, without affecting them in any visible way; (g) the non-filtrability of the etiological agent; (h) the chronic carrier stage; (i) the acquired immunity; (j) the cultures *in vitro;* (k) the transmissibility of the disease by ticks of the genera Boöphilus, Rhipicephalus, Ixodes and Dermacentor.

The clinical symptoms and postmortem pathology have been sufficiently described by various authors, Theiler, Sergent *et al.*, Giltner, and Stiles. Our findings are in substantial agreement with theirs and we shall therefore omit a redescription. There are only two points to which we wish to call attention, *viz.*, Sergent and his coworkers state that a subicterus is occasionally noticed; others consider the icterus as one of the most noticeable clinical symptoms, so much in fact that in the affected territory around New Orleans the disease is known as "yellow jaundice," and this term, while obviously redundant, expresses very well the layman's impressions.

In our experience the degree of icterus varies and the ease with which it can be observed varies with the color of the skin. In light-colored animals it is most readily observed in cows and heifers in the vagina and on the udder, and in bulls on the scrotum and on the skin between the hind legs. The ears also, when observed against the light, may show icterus. In dark-skinned animals the degree of icterus is indicated by the color of the eye.

The second point is the color of the urine. Most authors report that the urine remains clear; this has been our experience. In no case have we observed any hematuria or hemoglobinuria.

Sometimes the urine is a very deep yellow, due, presumably, to the presence of biliverdin. Gish,[21] reporting cases from Kansas, states that hemoglobinuria occurs occasionally in mature affected animals and may be common among young affected animals. Diarrhea is occasionally present, but constipation is found more usually. Points (c) and (j) will be considered together later. (d) The alterations in the blood also have been described often and in detail by various authors, and since nothing can be added it seems unnecessary to dwell upon them here at any length. Our findings agree with those of others, *viz.*, we have a tremendous anemia, poikilocytosis, anisocytosis, basophilic stippling, polychromatophilia, normoblasts, etc. Blood counts,* even in mild clinical cases, have shown a drop from the normal 6,000,000 to 7,000,000 erythrocytes per cmm. to 2,000,000 per cmm. and below.

In order to check (e), the impossibility of increasing the virulence by successive passage through susceptible animals, the following experiment was performed: A rather severe case of anaplasmosis occurred in the beef herd in cow 243. On October 24, 1928, the temperature was 105.1° F. and she presented all the clinical symptoms of anaplasmosis. The diagnosis was confirmed by blood-smear. The animal did not die. Steer 19 was injected December 17, 1928, with 30 cc of blood from cow 243. Anaplasma was found January 5, 1929, and the temperature rose to 104° F. on January 7, 1929. Steer 21 was injected on January 9, 1929, with 50 cc of blood from steer 19. On January 19, *i. e.*, 10 days after inoculation, the temperature rose to 103.6°F. Anaplasma was found in the blood in small numbers. Steer 22 was injected on January 21, 1929, with 50 cc of blood from steer 21. Anaplasma was found in steer 22 on February 13, 1929, and the temperature rose on February 15 to 104.4°F.

Summarizing the results on the three animals we have:

Steer 19: Quantity of blood injected, 30 cc; incubation time, 21 days.

Steer 21: Quantity of blood injected, 50 cc; incubation time, 10 days.

Steer 22: Quantity of blood injected, 50 cc; incubation time, 25 days.

In the cases of steers 21 and 22, the blood was taken at the height of the fever period.

An experiment was then undertaken to check (f), *i. e.*, the effects of the passage of Anaplasma through the goat and the sheep. The fact that the blood of a bovine suffering from anaplasmosis can be injected into a sheep without affecting the

*All blood counts mentioned in this paper were made by Dr. C. W. Rees.

sheep in any visible way whatsoever, and that after a certain time blood withdrawn from the sheep and injected into a susceptible bovine can produce anaplasmosis, is probably the most outstanding differential diagnostic characteristic of this disease. The following experiment was performed to see whether our disease agreed on this point with anaplasmosis as reported.

Two goats were injected with defibrinated blood from cow 19 on January 9, 1929. Blood was withdrawn from one goat into sterile sodium citrate on February 11, 1929, *i. e.*, after a period of 33 days. The blood was immediately injected into cow 23. There was no reaction for a period of 42 days. On March 25, 1929, blood was taken from cow 19, a carrier of anaplasmosis, and injected into cow 23, in order to determine whether the blood of the donor was infective and whether the recipient was susceptible. In the reaction which followed, Anaplasma appeared after an incubation period of 24 days. The temperature reaction occurred in 25 days. The passage through the goat, therefore, was not successful in this instance.

Two sheep were injected on February 18, 1929, with citrated blood from cow 22. One sheep was bled on March 21, 1929, and the blood injected into cow 28. A reaction followed in 34 days, *i. e.*, the blood showed Anaplasma on April 24 and a rise in temperature occurred on the same date. The results of these two experiments agree with results obtained by Sergent.

In order to determine whether the etiological agent belongs to the filtrable viruses, the following filtration experiment was tried: Animals 16 and 17 were bled, the blood was collected in sterile mason jars and allowed to clot. Twenty-four hours later, 50 cc of clear serum was drawn into a 50-cc syringe and injected into animal 27. About the same amount of serum was passed through a sterile Mandler filter No. 6. Owing to the small amount of serum and the large volume of the mantle surrounding the candle it was found necessary to add physiological saline to the serum. About 150 cc of filtrate (serum plus physiological saline) was injected intravenously into animal 26 on March 14, 1929. No. 27 reacted after an incubation period of 26 days. No. 26 showed a slight temperature reaction for four consecutive days from the 57th to the 60th day, inclusive, following inoculation. Anaplasma was found in small numbers. The blood appeared anemic and a count showed a drop to 4,000,000 r.b.c. per cmm. Anaplasma became more numerous on the 64th and 65th days and there was a further drop in the red blood cell count.

A second filtration experiment, using washed and laked red blood cells, was made. Blood was drawn from cow 23 at the time

when the stained smear showed marginal points in the red blood cells. About 350 cc of blood was drawn into a previously sterilized Erlenmeyer flask containing 35 cc of 20 per cent sodium citrate. This was allowed to stand for 48 hours and the supernatant liquid was pipetted off. About 6 cc of red blood cells was poured into 2 sterile 15-cc centrifuge-tubes. Sterile physiological saline was added to the 15-cc mark. The material was centrifuged and washed six times with sterile physiological saline. After the last washing, the supernatant saline was removed and sterile distilled water added to lake the cells. The contents of the centrifuge-tubes were filtered through filter paper to remove the residue after laking. Enough salt was added to bring the concentration up to 0.85, and 50 cc of salt solution was added to give volume.

The 80 cc of liquid was injected into cow 30, on April 24, 1929. Following this inoculation, the temperature of this animal oscillated between 100 and 103°F. for a period of 54 days, but at no time could anaplasmosis be diagnosed with certainty on the basis of the appearance of Anaplasma in the blood-smear. At times during this period Anaplasma-like bodies appeared in the smear but the irregularity of their occurrence and the small numbers in which they appeared did not justify a diagnosis of anaplasmosis.

To the remaining red cells in the flask, enough sterile physiological saline was added to restore the volume to about 300 cc. This was allowed to stand another 24 hours. The supernatant liquid was again removed and salt solution added to restore the volume. The red blood cells were thoroughly mixed with the salt solution and then distributed into 12 test-tubes. After being centrifuged and washed six times with physiological saline, the cells were laked with sterile distilled water. To the volume of laked blood enough salt was added to bring the concentration to 0.85. This was now filtered through a Mandler filter candle, previously sterilized, and 150 cc of filtrate was injected into cow 31 on April 25, 1929. The temperature record taken on this animal daily for a period of five months (April 26, 1929 to September 24, 1929) shows no deviation from the normal.

On September 23, 1929, cow 30 was injected with 45 cc of citrated blood from steer 27. Anaplasma was found in the blood on October 14, 1929. The temperature rose to 105.2° F. on October 18, 1929, and the animal died on October 21, 1929. Cow 31 was inoculated on September 22, 1929, with 45 cc of citrated

blood from animal 23. Anaplasma was demonstrated on October 14, 1929. The temperature showed only a slight elevation to 102.4° F., on October 20, 1929. The animal recovered. No definite conclusions can be drawn from this experiment. The infection of cow 26, 57 days after inoculation with the filtrate of a mixture of infective blood and physiological saline passed through a sterile Mandler filter candle, may have been accidental, but no explanation can be offered at the present time for the failure of cow 30 to react to the injection of hemolyzed blood of cow 23.

The chronic carrier stage (h): The question of the length of time an animal once recovered from an attack of anaplasmosis may remain a carrier is of decided interest and practical importance. An opportunity to obtain some information on this point was furnished us during the summer of 1928, through the courtesy of Dr. Collins, of Week's Island, La. Dr. Collins stated that one old dairy cow in the herd in his charge had passed through an attack of anaplasmosis three years previously, *i. e.*, in or about 1925.

This animal (cow 12) and two yearlings from their beef herd were transferred to the experiment barn in Jeanerette. Cow 12 was bled and 50 cc of her blood injected into animal 13Hd on September 4, 1928. Anaplasmosis developed on September 24, 1928; *i. e.*, after an incubation period of 20 days. This showed that the blood of this animal was infective almost three years after the recovery of the animal from an acute attack.

In an experiment to determine whether an animal once recovered from an attack of anaplasmosis is immune to further infection, the following animals were treated as noted:

Cow 12, as related, had passed through an attack of anaplasmosis in 1925. Her blood, injected into animal 13Hd on September 4, 1928, produced a reaction in 20 days. She herself was injected on the next day with 50 cc of blood from animal 7B. No reaction followed in 40 days. She was then returned to the owners.

Cow 1, which passed through an attack of anaplasmosis in 1927, was injected on February 18, 1929, with 25 cc of citrated blood from animal 22, at a time when Anaplasma could be demonstrated in the blood used for injection. No reaction followed in 90 days.

Animals 4 and 5 were similarly injected with blood from animal 22 on the same date. No reaction followed in a period of

31 days. These animals had to be sold in order to make room in the barn for animals for other experiments.

In order to compare anaplasmosis, as encountered by us in the form of pure anaplasmosis, with the anaplasmosis occurring in conjunction with piroplasmosis, we obtained some blood, containing both Piroplasma and Anaplasma, from Dr. H. Schmidt, of the Texas Agricultural Experiment Station. Animals 19, 20, 24, 25 and 29, used in this experiment, were injected with blood from Texas. Animals 19 and 20 had passed through an attack of pure anaplasmosis. No. 24 was a dairy cow, obtained from the dairy herd, maintained at this station. No. 25 was an animal obtained from Colorado. No. 29 was a yearling bull born and raised on the farm, but maintained apart from both dairy and beef herds. All passed through an attack of piroplasmosis.

Nos. 19 and 20 showed a slight recurrence of Anaplasma and a slight temperature reaction. Cow 24 showed no anaplasmosis reaction; she was probably immune. No. 25 died of piroplasmosis.

Bull 29 reacted to both piroplasmosis and anaplasmosis. Both reactions were mild.

We believe, therefore, that on the basis of these results, we are justified in concluding that the anaplasmosis occurring in conjunction with piroplasmosis in ticky territory is the same as the pure anaplasmosis occurring in tick-free territory.

An experiment to determine whether the red blood cells or the serum contains the etiological agent of anaplasmosis was as follows:

Blood was drawn from cow 22 into a flask containing 10 cc of a 20 percent solution of sodium citrate. The flask and contents and the bleeding needle were autoclaved before using, and 30 cc of citrated blood was poured into two sterile 15-cc centrifuge-tubes. The blood was centrifuged and washed six times with sterile physiological saline. At the end of this operation the centrifuge-tubes contained each about 3 cc of packed red blood cells. To each tube was added about 12 cc of saline. The contents of both were then poured into a beaker, drawn up into a 50-cc syringe and injected intravenously into animal 20, on February 18, 1929. Reaction took place on March 5, 1929, after an incubation period of 15 days. The reaction occurring in cow 27, following the injection of serum, serves as a counterpart to this experiment. The result of this experiment, taken together with the result of the experiment in which cow 27 was injected with serum alone, demonstrates that both the red blood cells and the blood serum contain the etiological agent of anaplasmosis.

ANAPLASMOSIS

IV. The Carrier Problem*

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TICK TRANSMISSION EXPERIMENTS

While the actual work reported on in this series of papers covers a period of two years, from 1927 to 1929, the review of the carrier and transmission problems should be carried beyond 1929 to 1932, for the sake of completeness.

Smith and Kilborne, in their paper entitled "Texas Fever," mentioned the occurrence of "peripheral cocci" or marginal points. Since they considered these "cocci" as a phase of *Piroplasma bigeminum* and since in their cases the only carrier of *Piroplasma bigeminum* was *Boöphilus annulatus*, they did not consider the possibility of a carrier of these "cocci" apart from *Boöphilus annulatus*.

Brumpt states:

> Aux Etats-Unis en 1893, Smith et Kilborne, ainsi qu'il resulte de l'étude de leurs expériences, n'ont jamais reproduit de "marginal points" en infectant leurs animaux avec des élevages de *Margaropus*. C'est tonjours par inoculation de sang ou chez des animaux vivant en liberté et infecté spontanément, qu'ils ont observé ces formes.

This statement that Smith and Kilborne never produced "marginal points" by infecting their animals through the medium of ticks raised in the laboratory, but that they observed these "points" only when animals were inoculated with blood (of carriers) and in animals spontaneously infected in the field is not correct.

An examination of the original paper of Smith and Kilborne shows the following: Leaving aside for the time being those experiments in which tick fever followed by "marginal points" was apparently transmitted by *Boöphilus annulatus* in the field or by blood inoculation, there remain 3 experiments, Nos. 12[*] and 13 performed in 1890 and No. 16 in 1891, involving a total of 14 animals, in which an attempt was made to transfer piroplasmosis by means of ticks raised in the laboratory. Two of the four animals in experiment 12 died of tick fever, one was killed *in extremis* 57 days after the ticks had been placed on it, and

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one recovered. In experiment 13, "an experiment to produce Texas fever in the winter season by placing young ticks on cattle, etc.," six animals (143, 145, 149, 117, 130 and 152) were involved; one of these (117) was a southern animal and according to our present interpretation it was immune. Nos. 143 and 149 did not react. No. 145 reacted to Texas fever on December 8, *i. e.*, 17 days after the seed ticks had been placed on her. On January 16, 1891, 56 days after ticks had been placed on her, the blood of this animal showed 15-20 per cent corpuscles infected with "peripheral cocci." No. 130 reacted to Texas fever 17 days after exposure; no "peripheral cocci" are mentioned. No. 152 also reacted to Texas fever 17 days after exposure and although no "peripheral cocci" are mentioned, the record shows that on February 18, 1891, *i. e.*, 67 days after exposure, the blood count dropped to 2,370,000 per cmm.

In experiment 16, which was a repetition of experiments 12 and 13, four animals were involved; three of these (166, 180 and 158) were susceptible, but the fourth (117) also used in experiment 13, was immune. All the experiment animals except No. 117 reacted to tick fever, No. 180 died and Nos. 166 and 158 recovered. No "peripheral cocci" are mentioned as occurring in either of the last two of these animals, but the records of both animals show a drop in red cell count due to tick fever. This drop, reaching its lowest point on the 24th and 28th days, respectively, following exposure, is followed by a rise in four days, which rise in turn is followed by a second drop 10 days later, and the second drop is followed by a slow rise after a period of several months.

Summarizing these experiments we have the following: Of 14 animals infested with seed ticks artifically raised in the laboratory, one used in two experiments was immune; two failed to react; and ten reacted to tick fever, three of these dying, one being killed *in extremis*, and six recovering. Of the six recovered cases, one showed "peripheral cocci," *i. e.*, on the basis of our present knowledge we would say that it reacted definitely to anaplasmosis; three of the cases show the changes in the blood count typical of the combined infection of piroplasmosis and anaplasmosis, although no "peripheral cocci" or marginal points are mentioned; two cases, although reacting to piroplasmosis, showed no "peripheral cocci" nor does the recorded blood count show evidence of anaplasmosis.

It is true that Smith and Kilborne suggest that in the one case (145) reacting positively to anaplasmosis, the infection may have

been derived from infective ticks matured on No. 117, a southern animal placed in the same stall with No. 145, rather than from the ticks placed on the animal, but the record shows that No. 145 reacted to tick fever 17 days after the first exposure to ticks, and therefore, long before the seed ticks placed on No. 117 could have matured, laid their eggs and produced a second generation of ticks. "Peripheral cocci" or marginal points appeared January 16, 1891, 56 days after the first exposure to ticks. That this appearance of "peripheral cocci" or marginal points could not have been due to ticks matured on No. 117, since November 21, 1890, is quite clear from the known facts of the life history of *Boöphilus annulatus*. Seed ticks placed on No. 117 on November 21, 1890, under the most favorable conditions, would have matured about December 10; with a preoviposition period of three days and a hatching period of 19 days, seed ticks would have emerged on or about January 1, 1891. Granted that these seed ticks might have infected cow 145 the same day, the period elapsing between the infestation with ticks and the appearance of "peripheral cocci" or marginal points would have been 15 days. Since Smith and Kilborne considered the "peripheral cocci" or marginal points as merely one phase of *Piroplasma bigeminum*, this period of incubation presented no difficulty to them, but on the basis of our present knowledge, this period of incubation of 15 days for the disease now known as anaplasmosis is much too short. We are, therefore, justified in drawing the conclusion at this time that in the case of cow 145, ticks hatched in the laboratory did transmit both piroplasmosis and anaplasmosis, with respective incubation periods of 17 and 56 days.

In addition to this one positive case and the three probable cases of anaplasmosis transmitted by ticks reared in the laboratory, the paper of Smith and Kilborne shows other cases of what is now known as anaplasmosis in northern or susceptible animals, after being placed in fields with southern animals infested with ticks, whereas in those cases where susceptible northern animals were placed in fields with southern (carrier) animals without ticks, no disease of any sort is reported. On the basis, therefore, of the data recorded by Smith and Kilborne, the conclusion that in their cases the cattle tick, *Boöphilus annulatus*, was the responsible carrier and transmitter of both piroplasmosis and anaplasmosis seems inescapable.

Theiler, in Part IV of his first paper describing anaplasmosis, published in the report of Government Veterinary Bacteriologist, 1908-1909, states that the "blue tick," *Margaropus decoloratus*,

transmitted both piroplasmosis and anaplasmosis. In a later paper,[4] published in 1911, he records some experiments by which he showed that both the "blue tick," *Margaropus decoloratus*, and the black-pitted tick, *Rhipicephalus simus*, transmitted the diseases in the larval stage.

Brumpt states that he was never able to transmit anaplasmosis to animals in France with seed ticks of *Margaropus calcaratus* and *Rhipicephalus bursa*, taken in Morocco, Algeria and Tunis on healthy animals, nor was he able to transmit the disease to animals in France with seed ticks of *Margaropus australis* originating in Brazil on healthy animals, although these seed ticks transmitted *Piroplasma bigeminum*, *Piroplasma argentinum* and *Spirochaeta theileri*.

Lignières reports that the tick *Boöphilus microplus* does not usually transmit anaplasmosis, and he suggests that some member of the genus Amblyomma may be a responsible transmitter.

Helm,[22] in Germany, reports that larvae of *Ixodes ricinus*, the castor-bean tick, hatched from eggs laid by adults fed on infected cattle, transmitted the disease to susceptible cattle.

Rosenbusch and Gonzalez[23] report successful transmission of anaplasmosis by *Böophilus microplus*, but only when the outside temperature is higher than 34° C. (93° F.). They report negative results with *Amblyomma sp.*

Sergent and co-workers[24] report the transmission of anaplasmosis by the ticks *Rhipicephalus bursa* and *Hyalomma lusitanicum*. Quevedo [25] reports that he has had opportunity to repeat, with analogous results, his previously (1916) successful experiments of transmitting anaplasmosis by means of larvae of *Boöphilus microplus*.

Rees[26, 27] reports the successful experimental transmission of this disease by nymphs of *Rhipicephalus sanguineus*, *Dermacentor variabilis* and *Dermacentor andersoni*, which were raised as larvae on clinical cases of anaplasmosis.

Brumpt[28] reports on some experiments performed by him in 1922 and 1923, in which he succeeded in transmitting anaplasmosis by means of larvae or seed ticks of *Margaropus annulatus microplus* after a period of 175 days and also by adults of *Rhipicephalus bursa*, raised as larvae and nymphs on a bovine infected with *Piroplasma bigeminum*, *Anaplasma marginale*, *Theileria mutans* and *Theileria annulata*.

Summarizing these results we have the following ticks incriminated as carriers or transmitters of anaplasmosis: *Boöphilus annulatus*, *Boöphilus decoloratus*, *Boöphilus microplus*, *Rhipice-*

phalus simus, Rhipicephalus bursa, Rhipicephalus sanguineus, Ixodes ricinus, Hyalomma lusitanicum, Dermacentor variabilis and *Dermacentor andersoni.*

Arthropods Other Than Ticks as Transmitters of Anaplasmosis

In view of the fact that, since the recent recognition of this disease in the United States, no ticks have been reported from field cases of the disease, with the exception of some cases in California, where specimens of *Ixodes ricinus* var. *californicus* and *Dermacentor occidentalis* were said to be present on the sick animals, suggestions have been made that biting insects may serve as possible transmitters of this disease.

Rosenbusch and Gonzalez, in 1927, examined *Haematopinus sp.* as a possible transmitter with negative results; similar results followed their attempt to transmit the disease by means of stable flies.

Our own "trial balloon" of placing a susceptible animal in a stall with an infected animal in the presence of mosquitoes and stable flies also gave negative results.

Brumpt expresses the opinion that biting flies, especially *Stomoxys calcitrans,* the ordinary stable fly, do not act as transmitters of this disease.

Sanborn and co-workers,[29] in the report of the Oklahoma Experiment Station for 1926-1930, report the successful transmission of anaplasmosis by biting flies of the family Tabanidae. The record, as given on page 260 of this report, shows that cow 21, the animal which subsequently developed anaplasmosis, was bitten on August 19, 1930, 38 times by three species of flies, *viz., Tabanus gracilis, Tabanus sulcifrons* and *Chrysops sequax,* 2 specimens of the last-named insect feeding once each on the carrier animal (No. 4) and once each on cow 21. A third specimen of *Chrysops sequax,* fed on the carrier (No. 4) only on that date, August 19, 1930. This insect was placed in a box and was subsequently allowed to feed on cow 21, on August 21, 22 and 25, 1930. In view of the fact that previous transmission experiments, reported in the same paper, with members of the genus Tabanus, resulted negatively, it appears that they are somewhat less probable carriers than *Chrysops sequax.* It is also probable that the transmission with flies, if it occurs, is a mechanical process, that is, there is no developmental stage of the parasite in the fly. Therefore, it appears that the feedings of August 21, 22 and 25 can also be eliminated as causative factors of the

later development of anaplasmosis in cow 21. This would leave the two bites of *Chrysops sequax* on both the carrier and the susceptible animal on August 19, 1930, as the actual transmitting factors. The long incubation period of 120 days favors this interpretation of the facts given in the report.

Transmission Experiments in Louisiana—1927-1929

In order to provide facilities for the transmission experiments and enable us to be reasonably sure that no possible extraneous carrier or vector intervened, a screened barn containing 10 stalls, 6 single stalls and 4 double stalls, was constructed. As may be seen from the accompanying floor plan (fig. 1), there were two screened doors to be passed through before the passage between the stalls was reached. Each stall was screened separately, so that any flies that might reach the inside of the stall could easily be killed.

Six genera and seven species of ticks are known to occur in Louisiana, *viz.*, *Boöphilus annulatus*, *Ixodes scapularis*, *Amblyomma americanum*, *Amblyomma maculatum*, *Rhipicephalus sanguineus*, *Hemaphysalis leporis-palustris*, and *Dermacentor sp.*

As the anaplasmosis cases called to our attention have occurred in areas officially declared tick-free, *i. e.*, free from *Boöphilus annulatus*, this tick need not be considered in our search for a possible intermediate host. Nevertheless, as this tick has never been proven experimentally to be a carrier of pure anaplasmosis, *i. e.*, anaplasmosis unaccompanied with piroplasmosis, in the United States, although tacitly accepted as such, it was thought advisable to obtain some experimental information on this point. The first batch of these ticks obtained in the laboratory consisted of some partially engorged females collected from a horse in East Baton Rouge Parish, La. These were sent by Dr. C. J. Becker. Only one of these survived and it laid about 200 eggs. The resulting larvae were placed on cow 7, a carrier animal transferred from Baton Rouge to Jeanerette on June 12, 1928. The ticks reached maturity about July 4, 1928. Several were collected and placed in test-tubes on moist sand. The ticks remaining on the animal were removed and destroyed. Oviposition began on July 7 and eggs hatched on July 25. Owing to the fact that the barn was not completed, we were compelled to wait until August 27, 1928, before the larvae could be placed on new subjects. On August 27, 1928, the larvae, which were now 33 days old, were placed on two new animals (8H and 9St). These ticks should have reached maturity about September 17, 1928, but only one

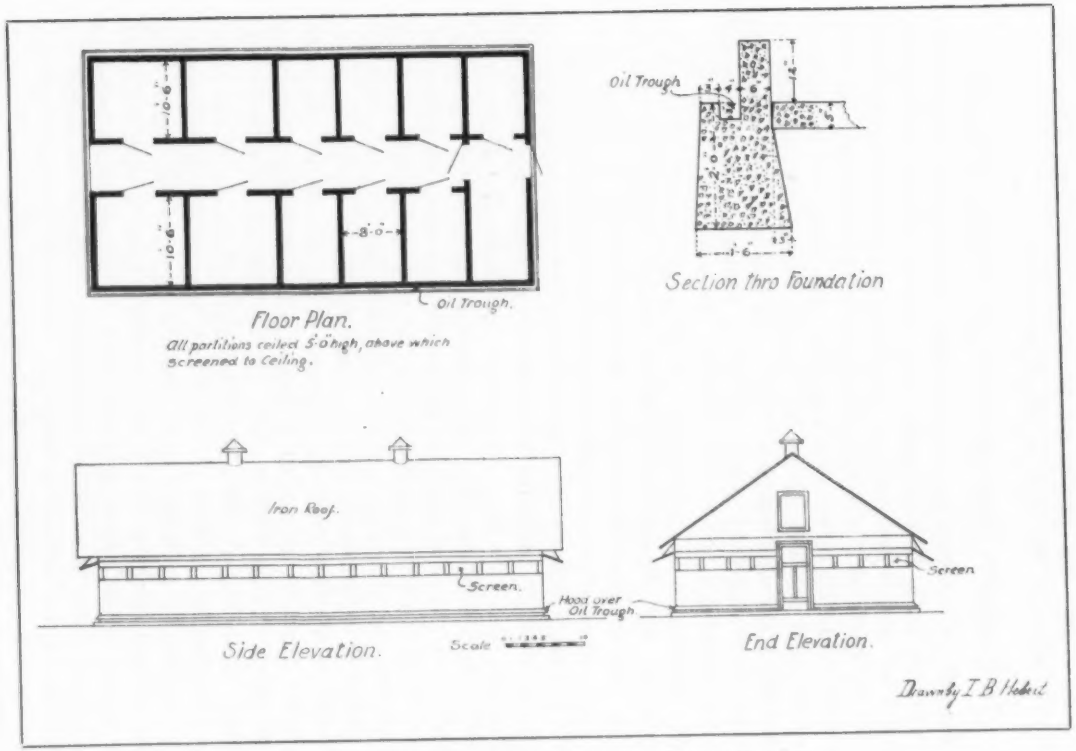

FIG. 1. Plan of experiment barn at Jeanerette, La.

adult was recovered from No. 8H on September 20, and only two were recovered from No. 9St on September 18.

On September 21 another batch of seed ticks was placed on these two animals. The ancestors from which these seed ticks were derived were taken from a horse at Opelousas, La., and forwarded to the laboratory by Dr. W. O. Ney, of Lake Charles, La. The ticks reached the laboratory on June 16, 1928. They were placed on clean, moist sand, where they oviposited, and the eggs hatched on July 7, 1928. The resulting larvae were placed on bull 4, inoculated intravenously on May 28, 1928, with 50 cc of blood from cow 1. (He reacted on June 18.) Several adult ticks were recovered from this animal, July 28 to 31. The remaining ticks were destroyed. The usual procedure was followed and eggs hatched about August 22, 1928. The resulting larvae were placed on animals 8H and 9St, September 21, 1928, and on animals 10C and 11H, on September 7, 1928.

Animal 8H reacted both with fever and parasites on November 19, 1928, *i e.*, after a period of 59 days. Animal 9St showed no reaction for a period of 126 days. Animal 10C showed no reaction to November 14, *i. e.*, a period of 68 days. Animal 11H showed no reaction until November 14, *i. e.*, a period of 68 days. Animal 10C was injected with 50 cc of blood from bull 4 on November 14 and showed a reaction on the 34th day (December 18), with a temperature of 104° F. in the morning and 106° F. in the afternoon for one day. Anaplasma appeared in the blood. Animal 11H received 25 cc of blood from bull 4 on November 14 and showed a typical anaplasmosis reaction December 3, an incubation period of 19 days. Animal 9St was injected with 45 cc of blood from bull 4 on January 25, 1929. No reaction followed within a period of 60 days; the animal, therefore, was considered immune and disposed of. Results: 1 positive reaction resulting from tick infestation; 1 absolutely negative reaction (11H); 1 doubtful reaction (10C); 1 immune.

A transmission experiment with *Amblyomma americanum* was as follows: A number of engorged females of *Amblyomma americanum* were obtained from East Baton Rouge Parish. The usual procedure was followed and the resulting larvae were raised to the unengorged adult stage on rabbits. The unengorged adults were placed on bull 5, injected on May 28, 1928, with 50 cc of blood from heifer 71, an animal in our dairy herd, which passed through a naturally occurring attack of anaplasmosis in November, 1927. (He reacted June 14, 1928, both with fever and parasites.) The adult ticks engorged and were removed from the

animal. The larvae of the next generation were placed on two animals (16 and 17) on October 25, 1928. They engorged in about 4 days and were collected. These animals showed no reaction for a period of 114 days following the application of the ticks. They were now injected (February 16, 1929) with 25 cc of blood from bull 5, the animal on which the adults had engorged. Both animals reacted promptly with fever and parasites on March 11, 1929, after an incubation period of 23 days. In this case *Amblyomma americanum* did not convey anaplasmosis through the eggs to the larvae and to susceptible cattle. Similar experiments were tried with *Ixodes scapularis* and *Rhipicephalus sanguineus*. The unengorged adults of these two ticks refused to attach and died.

REFERENCES

[21]Gish, A. H.: Anaplasmosis of cattle. Vet. Med. xxiii (1928), 3, pp. 106-109.
[22]Helm, R.: Beitrag Zur Anaplasmen problem. Zeit. für Infekt. Krankh., xxv (1924), 5, pp. 199-226.
[23]Rosenbusch, F., and Gonzalez, R.: Die Tristeza-Übertragung durch Zecken und dessen Immunitäts probleme. Arch. f. Protistkd., lviii (1927), 2, pp. 300-320.
[24]Sergent, E., et al.: Les Piroplasmoses bovines d'Algerie. Arch. de l'Inst. Past. d'Algerie, ii (1924), 1, pp. 1-146.
[25]Quevedo, J. M.: La Tristeza de los Bovines Piroplasmosis—Babesielosis—Anaplasmosis. Jesus Menendez. 186 B. de Irogoyen, Buenos Aires. (1929), pp. 1-196.
[26]Rees, C. W.: The experimental transmission of anaplasmosis by *Rhipicephalus sanguineus*. No. Amer. Vet., xi (1930), 9, pp. 17-20.
[27]Idem: Experimental transmission of bovine anaplasmosis and piroplasmosis by means of an infected lancet. No. Amer. Vet., xi (1930), 10, pp. 17-20.
[28]Brumpt, E.: Transmission d' *Anaplasma marginale* par *Rhipicephalus bursa* et par *Margaropus*. Ann. de Parasitol. Humaine et Comparée, ix (1931), 1, pp. 4-9.
[29]Sanborn, C. E., et al.: Transmission of anaplasmosis by flies. Rpt. Okla. Agr. Exp. Sta., 1926-1930, pp. 254-256.

Illinois State Fair in August

It is expected that attendance at the 1933 Illinois State Fair, at Springfield, will be helped by the Chicago Century of Progress, since the latter will have no live stock show. The fair will be held August 19-26, immediately after the A. V. M. A. convention. Premiums to be paid are expected to exceed any other fair by $50,000.

Handling Overproduction in Denmark

Over 10,000 cattle were slaughtered in Denmark during March, 1933, in order to limit production of beef and dairy products, and it is reported that the total number will reach 60,000 head. This device for controlling production is financed by a fixed tax on each head slaughtered for commercial purposes.

THE PERSISTENCE OF BRUCELLA AGGLUTININS IN CALVES OF REACTOR COWS*

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Many cattle-owners engaged in Bang's disease control† in Illinois have considered it expedient to retain temporarily one or more reacting cows in quarantine until calving time. In some herds, reacting cows have been rebred and isolated as calving time approached. This procedure is not recommended in Bang's disease control, but, as practiced on some farms, many heifer calves of good breeding lines from reacting cows have been raised free from Bang's disease. In connection with the suppression of Bang's disease in one herd, wherein several reacting cows were kept for a period of approximately five years, the agglutinin titre and the persistence of agglutinins in calves dropped by both non-reactors and reactors were observed. The tests of calves from non-reactor cows served, in some respects, as controls.

In the development and use of the agglutination test in the diagnosis of Bang's disease, different investigations on the origin and persistence of Brucella agglutinins in calves from reacting cows have been reported. Huddleson[1] reported that antibodies (agglutinins and complement-fixing) for *Brucella abortus* were not transmitted from the bovine mother to the fetus *in utero*, while Little and Orcutt[2] demonstrated the transmission of agglutinins for *Br. abortus* from cow to calf in the colostral milk. Huddleson and Halsey[3] further observed that antibodies (agglutinins and complement-fixing) for *Br. abortus* persisted in calves nursing reacting dams for periods varying from 48 to 125 days. The calves nursed their dams for periods varying from 3 days to 8 weeks (usually the nursing period was from 6 to 8 weeks).

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†Since 1922, the essentials of the plan developed by Barnes, of Pennsylvania, for Bang's disease control have been recommended in project form to Illinois dairymen and cattle-breeders by the Extension Service of the College of Agriculture. At this time, there are 790 voluntary coöperators located in 94 counties in Illinois. Sixty-two herds have been accredited by the State Department of Agriculture as free from Bang's disease, while 96 veterinarians have been accredited to make the preliminary herd tests. More than 20,000 cattle are under veterinary supervision and the program to date in Bang's disease control has been favorably received by Illinois breeders. Through the coöperation of the State Department of Agriculture, official quarantine is waived on reactors in herds enrolled in the Bang's disease control project. Reacting animals, however, are under the supervision of the local veterinarian and are isolated by the owner at time of calving to prevent intraherd dissemination of the disease. With relatively few exceptions, progress has been recorded in the suppression of Bang's disease on farms employing temporary isolation of reacting cattle. Retaining reactors in temporary isolation is not recommended, but is recognized as expedient under conditions where prompt elimination of reactors is not practiced.

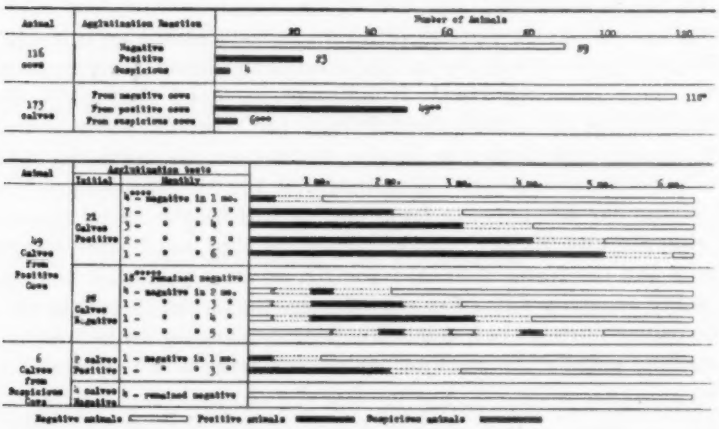
REPEATED AGGLUTINATION TEST OF CALVES

In the herd observed by the writers, a total of 173 calves were tested repeatedly at monthly intervals for Brucella agglutinins in determining their fitness to be transferred to clean premises. The first test was made 4 to 48 hours after birth.* The calves, with a few exceptions, were allowed to nurse their dams from 5 to 7 days. Then pasteurized milk was provided. During the five years, 118 calves were dropped by 89 negative cows, 49 calves by 23 positive cows, and 6 calves by 4 suspicious-reacting cows† (table I). All of the 118 calves from negative cows were negative at the initial test and remained negative. Of the 49 calves from positive cows, 21 were positive at the initial test and 28 were negative. Four of the six calves from suspicious reactors were negative at the initial test and two were positive.

PERSISTENCE OF AGGLUTININS IN CALVES FROM POSITIVE COWS

A. Calves positive at initial test: Of the 21 calves positive at the initial test, four become negative in one month. One of these

TABLE I—*The persistence of Brucella agglutinins in calves.*



*All remained negative.
**Twenty-one were positive and 28 negative at initial test.
***Two were positive and 4 negative at initial test.
****One calf died during the first month; three calves were destroyed before the second monthly test.
*****Three calves died during the first month.

*In the early tests, dilutions of 1:50 and 1:200 were employed, while an additional dilution of 1:100 was utilized as the work progressed. Calves were considered positive if the blood-serum reacted in any of the 3 dilutions. No grouping of suspicious-reacting calves was made.

†Cows were considered positive, if at any time during their breeding history either or both blood- and milk-serum had shown agglutination with Brucella antigen in dilutions of 1:200 or 1:100. Agglutination in dilutions of 1:50 in either or both blood- and milk-serum at any time during their breeding history was regarded as suspicious.

calves showed a 1:50 trace at the initial test and the three others reacted in dilutions of 1:200. Seven of the calves positive at the initial test showed Brucella agglutinins for two months but were negative at the third monthly test. One of the seven calves gave an initial reaction of 1:50 and gave similar reactions at the first and second monthly tests. The remaining six calves gave initial reactions of 1:200. Five of the six calves showed similar reactions at the first and second monthly tests while one calf reacted in a dilution of 1:200 at the first monthly test and 1:100 at the second monthly test.

Three of the 21 calves positive at the initial test became negative in four months. These three calves gave initial reactions of 1:200. Two of the calves reacted in 1:200 dilutions for three months. The other calf gave a 1:200 reaction at the first monthly test, was negative at the second, and gave a 1:50 reaction at the third monthly test. Two of the 21 calves, with an initial reaction of 1:200, continued to react in a dilution of 1:200 for four months. Another calf showed a 1:200 reaction on the initial test as well as the first, second and third monthly tests, followed by a 1:50 reaction at the fourth and fifth monthly tests.

Of the 21 calves positive at the initial tests, one calf died before the first monthly test was made, and three, that gave positive tests the first month, were destroyed before the second monthly test was made. Agglutinins persisted for one to five months (average of approximately three months) in the 18 surviving calves that showed positive initial reactions.

B. Calves negative at initial test: Of the 28 calves from positive cows negative at the initial test, 18 remained negative. Eight of the 18 calves that remained negative were from positive cows that did not show agglutinins in either milk or blood at calving time. One of the 18 negative calves was from a positive cow that did not show Brucella agglutinins in the milk at calving, and five of the nine negative calves from positive cows, that showed agglutinins in both blood and milk, did not nurse their dams. Three of the 28 calves died during the first month. Two of these calves had not nursed their dams prior to the initial test.

Seven of the 28 calves negative at the initial test became positive by the first or second monthly test. Four of the seven calves reacted in the first monthly test but were negative in the second monthly test. Two of these calves gave 1:50 reactions and two gave 1:200 reactions at the first monthly test. Of the remaining three of the seven calves that became positive after a negative initial test, one reacted in a 1:200 dilution at the first and second

monthly tests but became negative by the third monthly test. A second calf gave a 1:200 reaction at the first, second and third monthly tests and become negative by the fourth monthly test. The third calf gave inconsistent reactions for four months, showing negative reactions the first and third months and reactions in a dilution of 1:200 the second and fourth months, and negative the fifth month. Agglutinins persisted from one to four months (average of approximately two months) in the seven calves that gave positive reactions following a negative initial test.

PERSISTENCE OF AGGLUTININS IN CALVES FROM SUSPICIOUS REACTORS

A. Calves positive at initial test: One of the two calves showed an initial reaction of 1:50 and a negative reaction in the first monthly test. The other calf gave an initial reaction of 1:200 which was again the same at the first monthly test, a 1:50 reaction at the second monthly test, and then became negative by the third monthly test. Agglutinins persisted for one to three months (average of approximately two months) in the two calves that showed a positive initial test.

B. Calves negative at initial test: Four calves were negative at the initial test and remained negative.

SUMMARY

Calves from reacting as well as non-reacting cows in one herd harboring Bang's disease, that were allowed in the majority of instances to nurse their dams for a period of five to seven days, were tested repeatedly for Brucella agglutinins. The first test was made when the calf was 4 to 48 hours old. Calves were removed from the herd to disease-free quarters on the basis of two monthly negative tests.

One hundred and eighteen calves from negative cows were consistently negative to Brucella agglutinins. Twenty-one of 49 calves from positive reactors, and two of six calves from suspicious reactors, were positive on the initial test but became negative in six months or less. Seven of the 28 calves from positive cows, negative on initial test, became positive. All proved negative in 6 months or less.

REFERENCES

[1]Huddleson, I. F.: The transmission of antibodies (agglutinins and complement-fixing) from mother to fetus *in utero*. Corn. Vet., vii (1917), pp. 284-291.

[2]Little, R. B., and Orcutt, M. L.: The transmission of agglutinins of *Bacillus abortus* from cow to calf in the colostrum. Jour. Exp. Med., xxxv (1922), pp. 161-171.

[3]Huddleson, I. F., and Halsey, D. E.: The significance of *Bacterium abortus* antibodies (agglutinins and complement-fixing) found in the sera of calves at birth or after nursing. Mich. Agr. Exp. Sta. Tech. Bul. 66 (1924).

THE PATHOLOGY OF SPONTANEOUS LEUKOSIS OF CHICKENS*

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A blood dyscrasia of chickens which seem to be occurring with increasing frequency, often with resultant significant losses to the individual flock-owner, is the condition conveniently referred to as leukosis. It is a common practice of many observers engaged in the postmortem diagnosis of diseases of chickens to designate indiscriminately cases of genuine leukosis and cases of neoplasia in which this condition of the blood is not present, by such terms as "fowl leukemia," "lymphatic leukemia," and "big liver disease." These terms are inadequate, confusing and frequently erroneous. Their use is justified by tradition rather than by an appreciation of the fundamental concepts of the pathologic changes concerned. We shall attempt here to describe the morphologic aspects of leukosis of chickens, and to distinguish the condition from other diseases of chickens with which it may be confused.

MATERIAL STUDIED

During the last four years, we have had the opportunity to observe a considerable number of cases of spontaneous leukosis among chickens, and have succeeded in establishing a transmissible strain of the disease which is now in the fourth generation. Although many of the spontaneous cases were not suitable for detailed study, twelve were studied more or less completely, and in six, the blood was examined while the respective chickens were living. Incomplete examinations were made also of the blood of the six other chickens of the same group, although the fact that the examinations were made after death detracts somewhat from the value of these particular observations. The descriptions which follow of the various aspects of the disease are based on observations made on material obtained from chickens affected with the spontaneous form of the disease and not on data obtained from those that were affected experimentally.

REVIEW OF THE LITERATURE

The occurrence of leukosis of the domestic fowl that could be transmitted to healthy fowls of the same species was re-

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ported first by Ellermann,[1] in 1908. As a consequence of extensive observations, he concluded that there were three forms of spontaneous leukosis of chickens, some of which are accompanied by anemia. According to Ellermann,[2] leukosis of chickens may be myeloblastic, lymphoblastic or erythroblastic, and each variety is transmissible to normal chickens by the intravenous inoculation of blood or emulsions of organs of affected chickens. Ellermann[3] considered the three forms of leukosis as different manifestations of the same disease, and that the inoculation of material from a chicken affected with the myeloblastic form of the disease might result in the development of the myeloblastic, lymphoblastic, or erythroblastic form. It was Ellermann's opinion that the disease was infectious, and due to a filtrable, ultramicroscopic virus.

The most important of Ellermann's observations, namely, that leukosis of chickens is capable of transmission by the use of infective inoculums, has been confirmed by many workers, including Hirschfeld and Jacoby,[4] Schmeisser,[5] Andersen and Bang,[6] Furth,[7-11] Jarmai,[12] Engelbreth-Holm,[13] and Engelbreth-Holm and Rothe Meyer.[14] Although Hirschfeld and Jacoby failed in their attempts to induce the disease in healthy chickens by cell-free filtrates, Andersen and Bang, and more recently Furth, Englebreth-Holm, and Jarmai and his associates[15] have succeeded in this regard. There is therefore no longer any doubt concerning the transmissibility of the disease by a substance that is capable of passing through a filter.

Recent observations, particularly those of Furth, have definitely shown that we can no longer agree with Ellermann's conclusions that each of the three forms of leukosis he described, namely, lymphoid, myeloid and erythroleukosis, gives rise to definite leukemia and is transmissible. It is evident that the condition referred to by Ellermann as lymphoid leukosis does not induce a leukemic state of the blood and is not transmissible to healthy fowls; but is instead an aleukemic, neoplastic process which one of us (Feldman[16]) designated as lymphocytoma.

From this review the following facts seem to be established: (1) Leukosis of chickens is a definite entity. (2) Transmission is possible by whole blood, plasma, emulsions of tissue, or cell-free filtrates. (3) Histologically myeloblastic or erythroblastic cells may predominate, or a mixture of these two forms may frequently occur. (4) A leukemic state of the blood is usually evident. (5) The lymphoblastic, neoplastic condition frequently

confused with myeloid and erythroleukosis does not induce a leukemic state and is not transmissible.

Nomenclature and Definitions

Before proceeding with the description of the various morphologic aspects of leukosis of chickens, it is desirable to define the terms used to designate the two forms of the disease. Since many confuse the aleukemic, lymphoblastomatous condition, known as lymphocytoma, with genuine leukosis of chickens, this disease likewise will be defined.

We make no defense for the nomenclature selected other than that in our opinion this simple classification is adequate in designating the essential pathologic changes concerned in this group of diseases. To insist on terms to designate subdivisions of what are thought to be unusual varieties of leukosis is as objectionable as referring to all cases as "big liver disease." The name selected should be indicative, as far as is practicable, of the major characteristics of the condition and the histogenesis of the cell responsible for the state of leukosis.

Since leukosis denotes the presence in the circulating blood of excessive numbers of leukocytes or their parental forerunners, this term will be used in conjunction with words designating the character of the cell constituting the predominating feature of the particular form of leukosis under consideration.

Myeloid or myeloblastic leukosis: This refers to a condition in which there is a neoplastic-like hyperplasia of the granuloblastic elements of the bone-marrow, with subsequent entry of the blood-stream by excessive numbers of immature myeloblastic cells. The formation of heteroptic foci of myeloid cells may occur in the liver and marked leukemia with definite anemia results. The disease is transmissible to healthy chickens.

Erytholeukosis: This term designates a condition peculiar to chickens and is characterized by the vigorous proliferation of erythroblastic cells and the appearance in the blood of hemoglobin-free precursors of erythrocytes in such numbers as to constitute leukemia. There is usually evidence of severe anemia, and an intravascular accumulation of primitive erythroblasts occurs in the bone-marrow, spleen and liver. The condition is transmissible to other chickens.

Lymphocytoma: Lymphocytoma of chickens is a malignant neoplasm, the type cell of which is the undifferentiated lymphocyte. The lesions consist usually of pinkish gray to grayish white, diffuse masses or nodules in the various organs, the

viscera of the abdominal cavity being most frequently affected. The neoplastic cells have an extravascular relationship, leukemia does not occur, and the disease is not transmissible.

Besides lymphocytoma, a malignant neoplastic process infrequently occurs among chickens which we prefer to designate myelocytoma. This entity was described by Mathews,[17] who named the disease "leukochloroma." Mathews observed 36 cases among 3,938 chickens (0.9 per cent) examined during a period of four years. Myelocytoma is composed largely of diffuse collections of differentiated myelocytes situated extravascularly. The cytoplasm of the myeloid cell of myelocytoma is characterized by being filled with moderately large, globular granules which stain intensely with eosin. Mitosis is common. Tumorous foci occur in various situations of the body, often (according to Mathews) in association with the bones. Unlike myeloid and erythroleukosis, myelocytoma has not proved transmissible.

It should be pointed out that the foregoing definitions of what constitute myeloblastic leukosis or erythroblastic leukosis refer to typical uncomplicated cases in which the cell type is predominatingly either one or the other of these two forms. Not infrequently cases occur in which there is a mixture of myeloblastic and erythroblastic cells, and it occasionally happens that the blood picture in a given case may vary from time to time, during the period of observation. What is originally assumed to be an example of myeloid leukosis may eventually become erythroblastic, or the erythroblastic form may become myeloid.

The cases of leukosis in which a mixture of the type cell occurs or in which the character of the type cell changes from one to the other are uncommon. Either one type or the other prevails in the majority of cases and in our series of twelve cases ten were erythroblastic, and in two cases erythroblastic and myeloblastic cells were present in approximately equal proportions.

INCIDENCE

The possibility of confusing lymphocytoma with myeloid and erythroleukosis, and the failure to recognize the former as a separate entity, have resulted in the grouping of these conditions by many observers under the one heading of leukemia of chickens. Consequently most of the reports in the literature are of doubtful value in disclosing the frequency of occurrence of the real disorder. When one becomes familiar with the salient features of lymphocytoma, myelocytoma and leukosis, a differential diagnosis usually is possible from an examination of the

gross lesions. To separate myeloid leukosis from erythroleukosis usually requires microscopic examination of the blood or histologic examination of the liver and spleen.

Our series of twelve cases was obtained from a group of approximately 100 chickens, aged nine months or more, on which necropsy was done to ascertain the cause of death. Many of the chickens were in a sick or dying condition when first observed, and when leukosis was suspected the blood was examined if possible. We have no information as to the total number of chickens in the flocks from which the chickens were obtained for necropsy. Probably 10 to 15 per cent of all deaths of adult chickens are due to leukosis, although authentic data are not to be had. In the absence of sufficient data on the incidence of leukosis of chickens it is sufficient to say that the disease is by no means uncommon, and next to lymphocytoma is one of the most common of the specific diseases of chickens due to causes other than pathogenic bacteria.

Relation to Breed, Age and Sex

Although no breed of chickens can be considered immune to leukosis, some breeds appear to be definitely more susceptible than others. In ten of the twelve spontaneous cases that we studied in detail the chickens were Barred Rocks, and two were White Leghorns. The majority of spontaneous cases observed by other investigators likewise occurred in these two breeds. One should be cautious in concluding that one breed is more susceptible than another since sufficient authentic data on this phase of the problem are not to be had. It seems significant, however, that while the flocks supplying most of our cases consisted of 300 to 400 chickens of the White Leghorn breed, from which two with leukosis were obtained, that ten cases should be encountered in a flock of Barred Rocks that usually average between 50 and 100 chickens. Stubbs and Furth[18,19] noted that some breeds were more resistant than others to the transmissible form of the disease. Briefly, their results were as follows: Of six Barred Rocks inoculated, four acquired leukosis; of six White Leghorns inoculated, four were affected; of six Rhode Island Reds inoculated, two were affected, and of six chickens of mixed breed inoculated, only two acquired the disease. Six Bantams were inoculated and all acquired the disease. The experimental observations of Stubbs and Furth indicate a variability in the inherent resistance of different breeds to the

disease, which is also apparent in the disease as it occurs spontaneously.

Spontaneous leukosis does not affect chickens during the first few months of life. In our series the age of most of the chickens was approximately one year, the youngest being nine months, and the oldest being twenty months.

There is no evidence that the disease exhibits a predilection for one sex more than another. Although it would seem that more hens than cocks are affected, this can be explained by the fact that more hens than cocks are permitted to reach maturity.

SYMPTOMS

The symptoms of leukosis usually vary with the severity of the attack, and none is pathognomonic. Early in the disease there may be a definite and characteristic immunity of either the erythrocytes or the leukocytic granulocytes associated with a normal exterior appearance of the fowl. As the disease progresses, and the number of hemoglobin-bearing cells in the blood is lowered, paleness of the comb and the wattles is noted. If one punctures the wing vein at this later stage of the disease the blood appears thin and watery and does not clot readily.

Depending on the duration of the disease there may be marked loss of weight. One chicken observed early in the disease weighed 1,613 gm, and at the time of death, three months later, after a chronic course, it weighed 860 gm. In acute attacks of leukosis, the loss of weight is scarcely noticeable, and cannot be accounted for by lack of consumption of food, for, as a rule, the appetite remains good.

In the chronic phase of the disease there is gradual muscular weakness, and in the late stages the chicken is unable to stand. This muscular weakness is not to be confused with a neuropathic paralysis. In the acute attacks, in which death occurs soon after the inception of the disease, there may be the same muscular weakness evidenced just prior to death.

The attitude of the affected chicken may vary. Some appear to be normal as regards excitability and response to the stimulus of fright. Others manifest general debility and dullness; they stand with feathers ruffled, neck retracted, eyes closed, and when disturbed seem to arouse sluggishly as from a stupor (fig. 1).

In general there is little that serves to characterize the disease from the standpoint of symptoms. In the absence of the necessary materials to make and study a stained blood-smear,

perhaps the best method of diagnosis is to puncture the wing vein and observe the blood for relative paleness, watery state, and poor coagulability. This, together with the general symptoms displayed and the gross pathologic changes present at necropsy, should lead one to the correct diagnosis of leukosis. It must be borne in mind that chickens may have secondary anemia, which can be distinguished by a more detailed study of the cytology of the blood. Often, however, the cause of secondary anemia may be apparent at necropsy and the condition thus can be distinguished from leukosis.*

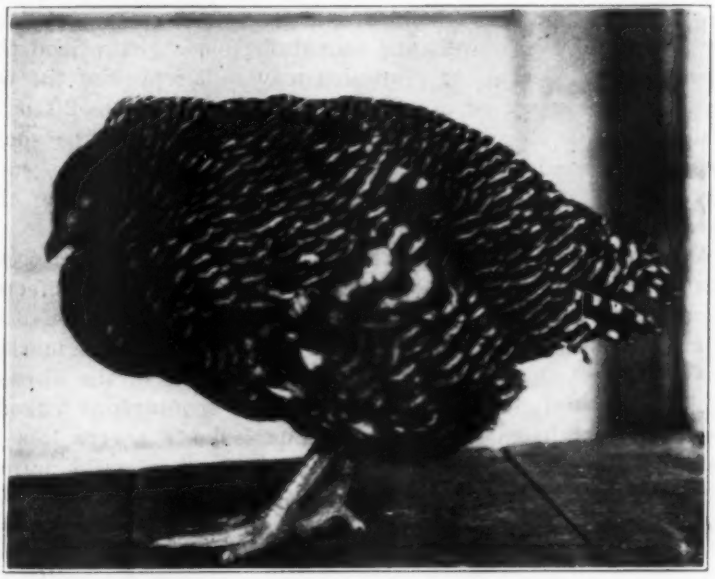

FIG. 1. Typical attitude of a chicken affected with leukosis.

COURSE OF THE DISEASE

Complete data on the course of spontaneous leukosis are difficult to obtain. The earlier stages usually are not observed, because there is nothing to distinguish the inception of the disease so that it can be detected by observation of a suspected flock.

*Since this paper was written, Stubbs and Furth's contribution (Stubbs, E. L., and Furth, J.: Anemia and erythroleucosis occurring spontaneously in the common fowl. Jour. A. V. M. A., lxxxi (1932), n. s. 34 (2), pp. 209-222) has appeared, in which the relationship between erythroleukosis and spontaneous severe anemia is considered. There appear to be factors involved in these two conditions which are common to both. Further study will be necessary, however, before the exact relationship of these conditions is understood.

In one case of spontaneous erythroleukosis, observed during the entire period of the disease, erythroblastic cells were present in the blood for 22 weeks preceding death. The erythrocytes and the hemoglobin gradually diminished. During the three weeks prior to death, the erythrocytes numbered less than 1,000,000 for each cubic millimeter of blood, and the hemoglobin content was 2.65 gm or less for each 100 cc of blood. This is typical of the chronic form of leukosis.

There may be periods of apparent recovery as judged by the examination of the blood. Erythocytes and hemoglobin content may increase and there will be fewer immature cells. The mature red cells and hemoglobin eventually return to the previous low levels and even lower, and immature forms again flood the blood-stream. Periods of remission may well represent the beginning of recovery, or of temporary resistance of the diseased tissues. Although actual and definite recovery from the spontaneous disease has not been reported, to our knowledge, such may happen, since recovery from the experimental form of the disease has been reported.[8, 12]

A chronic and an acute form of the disease appear to occur when chickens are inoculated experimentally with the affected blood and the same appears to be true with spontaneous leukosis. In acute attacks produced experimentally there is not the marked loss of weight that occurs in the chronic forms of the disease. Such loss of weight is observed in chronic spontaneous leukosis also. In certain cases of spontaneous leukosis severe loss of weight does not occur, and the chickens appear quite normal until suddenly they become very pale, and death soon follows. This condition is no doubt comparable to that of acute leukosis of chickens inoculated experimentally.

GROSS PATHOLOGIC ANATOMY

The organs most commonly affected with leukosis are the liver, spleen, bone-marrow, kidneys and intestines.

The liver usually is enlarged, although occasionally its weight will be within normal limits. The weight of the liver may bear a significant ratio to the weight of the body. For example, in one case the body weighed 2,400 gm and the liver 360 gm, with a ratio of 1:6.66 as the weight of the liver to the weight of the body. Latimer[20] reported a ratio similarly calculated as 1:52 for normal adult chickens. The color of the liver varies considerably. It is usually red brown, with an occasional variation to a lighter or darker shade. Almost always small pin-points

of a creamy or yellow color are to be seen scattered indiscriminately over the surface of the organ (fig. 2). Occasionally the capsule of the liver will rupture, with hemorrhage into the peritoneal cavity. In two of the chickens of our series a subcapsular blood clot was found, as evidence of hemorrhage at some previous time.

The spleen always is enlarged to several times its normal size. Latimer,[20] in a study of 100 normal adult chickens, gives

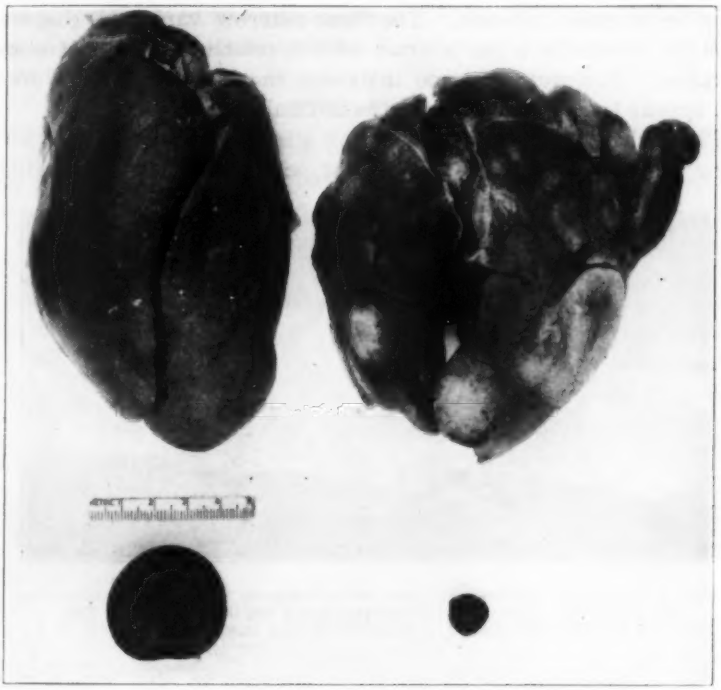

FIG. 2. The liver and spleen on the left are from a chicken affected with spontaneous erythroleukosis. Specimens on the right were obtained from a chicken affected with lymphocytoma. The difference in the sizes of the respective spleens is significant. The presence of multiple nodules of neoplastic tissue in the liver on the right is characteristic of lymphocytoma of chickens.

the average weight of the spleen of a mature chicken weighing about 2,600 gm as 2.73 gm. In leukosis it is not uncommon to find spleens weighing 20 to 40 gm. The largest spleen in our series weighed 44 gm, as contrasted to the weight of the body of 2,010 gm. The consistence of the organ is somewhat softer than normal and the color usually is reddish violet. On cross-

section the substance of the spleen is homogeneous and with no suggestion of focal accumulations of immature cells. When the spleen is cut in the middle, transverse to the long axis, there is bulging of the cut surface and rolling outward at the capsular margin, as though there were increased pressure from within.

The color of the bone-marrow varies from a light yellowish red to a dark purple-red. There appears to be a relative thinning of the bone of the femur, as it seems to cut with more ease than in normal chickens. The bone-marrow varies in consistence; it is usually a pasty mass with a relative absence of bony lamellae. However, in some instances the marrow grossly does not appear to deviate far from the normal.

The kidney is not constantly the site of gross lesions. The color of the organ varies from light yellowish brown to reddish

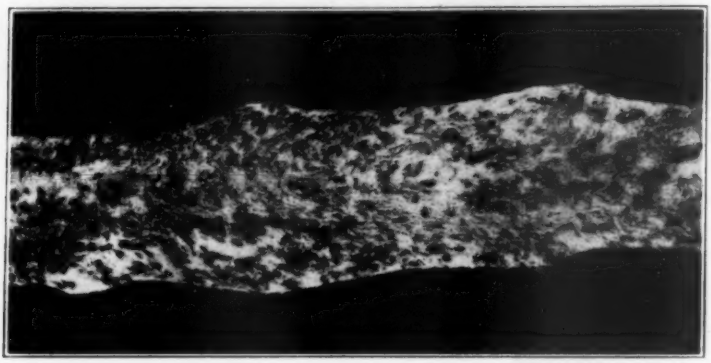

FIG. 3. Multiple hemorrhagic foci in the mucosa of the small intestine of a chicken affected with experimental erythroleukosis. The chicken died 155 days after the inoculation.

brown. There may be an apparent increase in size, which may be uniform or confined to either the anterior or posterior poles. In a few cases there may be small pin-point, creamy or yellow foci scattered throughout the organ.

Because of the poor coagulability of the blood, the result of thrombopenia, hemorrhages are seen commonly at necropsy. The mucosa of the duodenum often is hemorrhagic, and the lesions become profound (fig. 3). When the hemorrhage is marked, the mucosa of the entire course of the intestinal tract is involved. However, the degree of the hemorrhage decreases toward the terminal portion of the intestine. Hemorrhages of the mucosa may be petechial or diffuse ecchymosis. Small hemor-

rhages may be seen in the subcutaneous and intermuscular areolar tissues. As mentioned, fatal hemorrhages also may occur from the liver into the peritoneal cavity from rupture of the capsule.

The thymus glands on each side of the neck may or may not show alterations. Occasionally a few of the glands of the thymus may be slightly enlarged and hemorrhagic.

A summary of the gross pathologic changes in leukosis would include an enlarged liver of brown to red color, dotted with small cream-colored foci; a spleen enlarged to four or five times its normal size; a pulpy bone marrow of reddish color, and hemorrhages into the subcutaneous tissues and mucosa of the duodenum.

THE BLOOD

In a consideration of the blood of chickens with leukosis, one must have in mind as a basis for comparison what constitutes the normal blood of chickens.

Scarborough,[21] in a recent review of the literature, noted the averages of the blood cell counts of the domestic fowl. These are given in table I.

On the basis of the blood picture, leukosis of the chicken may be separated into two types, with a third type of more or less arbitrary differentiation. These are erythroleukosis, myeloid leukosis and a mixed form in which immature forms of both the erythrocytic and granulocytic series of cells abound in almost equal

TABLE I—*Comparison of normal averages of the respective elements of the blood reported by Scarborough and averages of six spontaneous cases of erythroleukosis.*

ELEMENT	NORMAL	ERYTHROLEUKOSIS
Erythrocytes	3,440,00	778,000 (590,000 to 1,180,000)* for each cmm
Hemoglobin	10.38 gm for each 100 cc	1.97 gm for each 100 cc
Thrombocytes	45,500 to 55,200	5,700 (700 to 13,250) for each cmm
Total leukocytes	25,900 (20,000 to 40,000)	24,000 (10,000 to 39,000) for each cmm
Lymphocytes (%)	51.6	71.7
Polymorphonuclears(%)	31.1	18.9
Eosinophils (%)	5.9	1.7
Basophils (%)	2.6	1.6
Monocytes (%)	10.0	5.5
Total erythroblasts	Very few	51,000 (17,000 to 126,000) for each cmm
Total myeloblasts	Rare	600 (200 to 1,000) for each cmm

*Figures in parenthesis indicate the ranges of variation.

numbers (fig. 4). In either erythroleukosis or myeloid leukosis, immature forms of the other series usually appear. In some chickens the transition of erythroleukosis to myeloid leukosis has been observed. Consequently every graduation between the two forms may appear. However, erythroleukosis seems to occur much more commonly than does myeloid leukosis. The pathologic or immature cells appearing in the blood in erythroleukosis represent every form from the mature erythrocyte back to the lymphoid cell of Furth.

The various stages of the development of the erythrocyte can be recognized and classified in the following descending order as propounded by Furth. The staining reactions are those obtained by Wright's method.

1. Erythrocyte is the normal, adult, hemoglobin-bearing cell of the blood. In leukosis, this cell assumes various bizarre forms, being spindle-shaped to spherical. The number of these cells in the blood drops to very low levels in the course of either type of leukosis.

2. Polychrome erythrocyte is characterized by tending to be more spherical than oval, and the basophilic zone of cytoplasm near the cell margin surrounds a well-marked zone of hemoglobin in the cytoplasm immediately about the nucleus. The nucleus is larger than in the mature erythrocyte. This cell is not uncommonly seen in normal blood.

3. Polychrome erythroblast is a cell only occasionally seen in normal blood. It is larger than its successor, the polychrome erythrocyte. The basophilic margin of the cytoplasm is of a slightly deeper tint and much broader. The perinuclear zone of hemoglobin is narrow and often only faintly discernible. The nucleus is larger in proportion to the size of the cell. Binucleated forms often are seen in the blood.

4. Lymphoid erythroblast is not encountered in normal blood. This cell is large and irregular, although tending toward spherical. The cytoplasm is narrow and basophilic, appearing to be finely granular. Often a paler staining perinuclear zone is evident. Mitotic figures often are seen in this stage of the developing erythrocytes; in many, vacuolization of the cytoplasm may appear.

The cell preceding the lymphoid erythroblast is usually evident in leukosis. Furth applied to this structure the term "lymphoid" cell. This cell is larger than its successor, the lymphoid eythroblast. The ratio of nucleus to cytoplasm is almost the same, but with slightly more cytoplasm in the major-

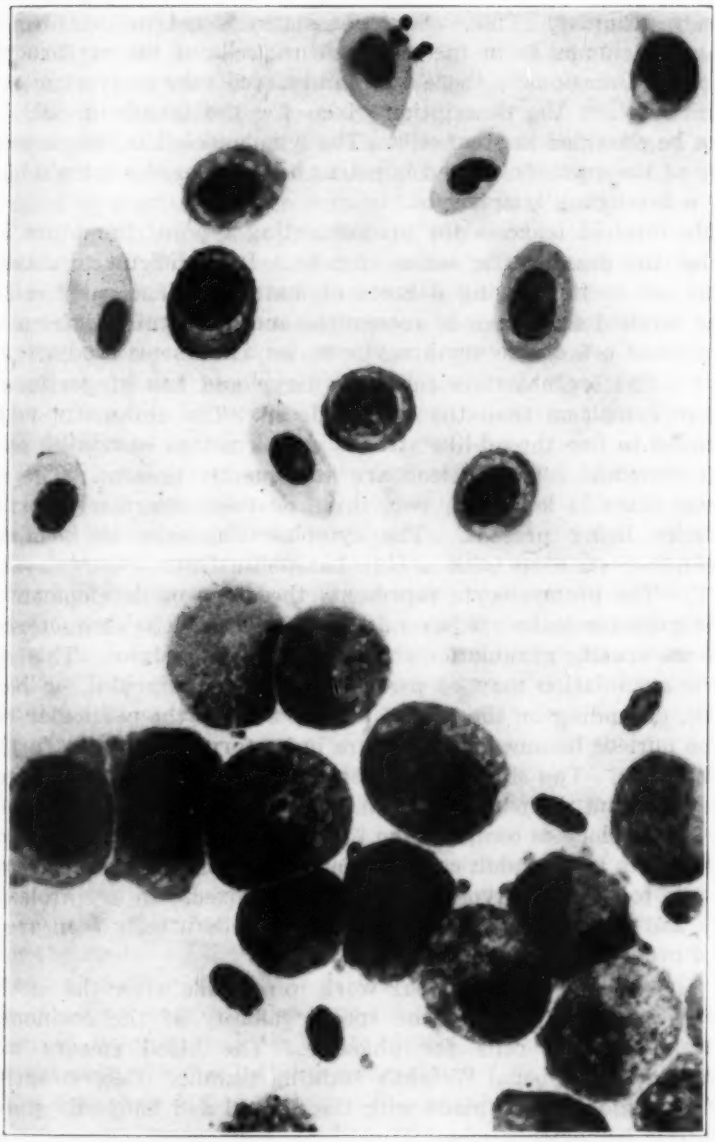

FIG. 4 (above). Several erythroblasts in blood-smear prepared from a case of spontaneous erythroleukosis. The preparation was stained by Wright's method (x 1350).

FIG. 5 (below). Numerous immature myeloblastic cells in a blood-film prepared from a case of experimental myeloid leukosis (x 1350).

ity of the cells. The cytoplasm is slightly less basophilic and finely granular. The nuclear chromatin is not in the form of angular clumps as in the more mature cells of the erythrocytic series. Occasionally there will be observed very early stem cells which follow the description given for the lymphoid cell, but can be classified as stem cells. The lymphoid cell is the progenitor of the erythrocyte and is not to be considered a lymphoblast or a developing lymphocyte.

In myeloid leukosis the predominating type of immature cell is of the granulocytic series (fig. 5). It is difficult to classify this cell in its varying degrees of maturity. The early cell of the myeloid series can be recognized and distinguished from the lymphoid cell of the erythrocytic series after some study.

1. The leukoblast is relatively large and has proportionally more cytoplasm than the lymphoid cell. The chromatin is arranged in fine thread-like strands and is not so basophilic as in the lymphoid cell. Nucleoli are infrequently present. The nucleus often is lobulated, two, three or four irregularly formed lobules being present. The cytoplasm appears to be finely granular, staining with a blue basophilic tint.

2. The promyelocyte represents the stage of development of the granular leukocyte beyond the leukoblast. It is characterized by the specific granulation coming into the cytoplasm. This specific granulation may be pseudo-eosinophil, eosinophil, or basophil, depending on the line of development of the particular cell. The nucleus becomes more mature in its form, tending to further lobulation. The chromatin becomes coarser with more differentiation from the parachromatin.

3. Myelocytes complete the line of development from the promyelocyte to the adult cell, and forms ranging from the promyelocyte to the adult type cell can be recognized. In erythroleukosis and mixed leukosis the myeloblastic cells usually seen are of the promyelocytic and myelocytic stage.

The cellular counts in our work were made after the method of Wiseman,[22] utilizing the specific affinity of the eosinophile granule-bearing cells for phloxine. The blood smears were stained by the usual Wright's staining technic. Determinations of hemoglobin were made with the Sheard and Sanford[23] photoelectric hemoglobinometer.

In leukosis the erythrocytes are greatly reduced; usually to less than 1,000,000 for each cubic millimeter of blood when the disease is first observed and in some cases less than 500,000 shortly before death. The hemoglobin content is correspond-

ingly low. In the six spontaneous cases of erythroleukosis in which the blood was examined shortly before death, an average of 1.97 gm of hemoglobin for each 100 cc of blood was found, which is a marked contrast to 10.38 gm for each 100 cc of blood, as reported for normal by Scarborough. Transmission experiments disclose that the degree of anemia may reach similar low levels in experimentally induced myeloid leukemia. The degree of anemia tends to parallel the course of the disease.

The immature cells appearing in the circulation is the first indication of the disease process. In erythroleukosis the stage of development of the immature cell predominating in the blood may vary, but as a general rule in the early stages the polychrome erythrocytes and polychrome erythroblasts are to be found in greater numbers than the lymphoid erythroblasts and lymphoid cells. However, all stages of the developmental process can usually be found (fig. 4).

In the acute attacks, or shortly preceding death, an abundance of lymphoid erythroblasts and lymphoid cells of Furth are seen, many with mitotic figures.

A few myeloblasts usually are seen in erythroleukosis; usually from 200 for each cubic millimeter of blood to almost as many as the number of erythroblasts, in which case the term mixed or myeloid leukosis would be applied. However, there usually are but few myeloblasts. In the six cases of erythroleukosis studied, the average number of myeloblasts was 600 for each cubic millimeter.

The total number of erythroblasts in the blood varies with the stage of the disease. Usually there is a progressive increase up to the time of death. In the six cases of erythroleukosis studied, the erythroblastic cells varied from 16,000 to 126,000 per cmm.

The thrombocytes or blood platelets are greatly affected. During the course of the disease there is a marked decrease in the number of these cells, which leads to poor coagulability of the blood. This may well account for the numerous hemorrhages seen at necropsy. Just before death the thrombopenia may be extreme. In one instance the estimated number of thrombocytes was only 700 per cmm. The average number in the six cases of erythroleukosis studied was about 6,000 per cmm as compared to Scarborough's 45,500 per ccm for the normal chicken.

In general the total number of mature leukocytes is not greatly altered in erythroleukosis. However, the differential count of mature leukocytes does exhibit a deviation from the normal.

The percentage of lymphocytes is increased, whereas the other cells are decreased. Table I gives a general idea of the changes that occur in erythroleukosis. In acute myeloid leukosis, as observed experimentally, few mature polymorphonuclear and eosinophilic granulocytes are to be found.

PATHOLOGIC HISTOLOGY OF ERYTHROLEUKOSIS

The fact that the cellular elements, which characterize erythroleukosis, are generally limited to the vascular spaces minimizes the structural alterations observed as a consequence of the prodigious overproduction of these cells. While extravascular accumulation of immature erythroblast cells may occur occasionally, this is an exception and the essential histopathology usually consists in a marked increase of immature cells in the various channels of the blood.

The immature erythroblastic cells, when stained with hematoxylin and eosin, are characterized by a large, coarsely granular basophilic nucleus and a narrow rim-like cytoplasm that stains acidophilic. Mitosis and other evidences of immaturity such as hyperchromatic nuclei are usually apparent. The histopathology of the various organs will be considered briefly.

Liver: Aside from the blood-stream itself, the liver offers the best opportunity for observing the specific changes characteristic of erythroleukosis. In a typical case the larger vessels will reveal an enormous increase in the number of erythroblastic cells in various stages of development, and occasionally the number of mature erythrocytes are so outnumbered as to constitute only a small percentage of the total cellular elements within the vessel. The channels of the capillary network are frequently gorged to capacity with unripe cells and it is not unusual to observe a liver in which a considerable amount of hepatic parenchyma has become atrophied, due to the constant and increasing encroachment of the hepatic cells by the dilated capillaries (fig. 6). Regardless of the degree of dilation attained by the capillary vessels, the ever-increasing erythroblastic cells retain their intravascular relationship and seldom accumulate outside of the blood-stream.

We have examined livers in which the capillary network of the organ was almost devoid of unripe erythroblastic cells, but in which there was unmistakable evidence of erythroleukosis in the larger vessels of the organs. In instances of this kind the examination of direct blood-smears is of the greatest value in ascertaining the true condition. It is not uncommon to find

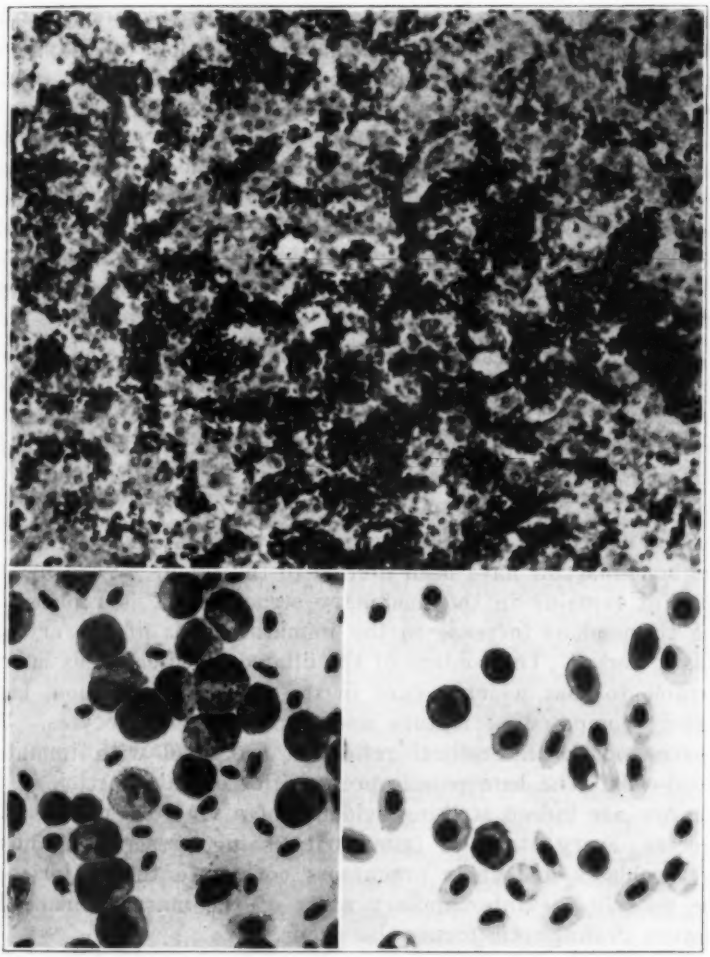

FIG. 6 (above). Liver of a chicken which died as a consequence of spontaneous erythroleukosis. The marked engorgement of the capillary spaces with immature erythroblasts is characteristic (x 220).

FIG. 7 (below). Blood-films of chickens affected with leukosis showing significant differences between the myeloid and erythroblastic forms of the disease: left, myeloid leukosis; right, erythroleukosis (x 540).

in livers from chickens affected with erythroleukosis small, irregularly distributed areas of coagulation necrosis, although aside from atrophy of some or many of the hepatic cells other severe retrogressions due to the condition usually are absent.

Spleen: The extremely vascular composition of the spleen permits of marked engorgement by the unripe blood-cells, which are frequently present in such numbers as to obliterate all structural details of the pulp tissues. The splenic corpuscles and the larger vessels remain intact, although the former often are atrophic. Although occasionally the outlines of a cavernous vein may be delineated definitely on account of the excessive cellular contents, these structures usually are not discernible. If the spleen is greatly enlarged, the septums are widely separated and thinning of the capsule occurs.

Kidney: Significant morphologic alterations of the kidneys do not occur, as a rule, in chickens affected with erythroleukosis. Occasionally, if the leukosis is particularly severe, the interstitial capillaries become distended with immature cells, but the capillary engorgement is never so marked as that which usually obtains in the liver and spleen.

Bone-marrow: Our observation of the anatomic changes of the bone-marrow have been limited to the femur. Very little if any fat remains in the medullary structure; it is replaced by the tremendous increase in the immature cells of the erythroblastic series. The content of the dilated blood-channels is comparable to that which occurs in the general circulation, being largely composed of mature and immature erythrocytes. The meshes of the interstitial reticulum are filled with immature blood-cells. The hemopoietic propensities of this portion of the marrow are indeed striking evidence of a vigorous proliferative process, every stage of immaturity being present. Although erythroblasts and their precursors constitute the majority of the cells in the intercapillary areas of the marrow, many immature granulocytic forms also occur.

The bony lamellae seem gradually to disappear and in serious forms of the disease these structures usually are absent.

Intestines: The occurrence of severe hemorrhage into the lumen of the intestines, in many cases of erythroleukosis, would suggest alteration of tissue. Microscopically the most significant lesions in the small intestine are found where the delicate capillary channels of the villi are greatly congested and where extravasation of blood is frequent because of the fragility of these

structures. A villus often is observed, in which there has been a tremendous influx of immature erythrocytes, with consequent dilation of many of the capillaries and rupture of others. The villus with its associated cells frequently become a structure several times normal size. The enlargement is due to elongated masses of cells being retained as single units by the reticulated remains of the villus and an external coating of mucus. Occasionally such a villus becomes detached and is found free in the lumen of the intestine, a mixture of mature and immature erythrocytes held together by a delicate matrix that was once the stroma of a villus. The cells of such a detached bit of tissue usually remain intact and individual cells separated from the parent mass seldom are seen.

In the smaller lumen of the cecum, the mucosa may become so hyperplastic as practically to close the lumen. The increase in the volume of the mucous membrane is due to enormous infiltration of the capillary bed and the adjacent tissue spaces by the erythroblastic cells conveyed there by the blood-stream, many of which not infrequently show evidence of necrobiosis.

Other tissues: Sections of the heart, thymus, lung, pancreas and ovary seldom reveal significant changes other than dilation of the capillary vessels as a consequence of engorgement with primitive erythroblastic cells. Focal accumulations of cells in these organs have not been observed in our material, nor has the leukosis been associated in these organs by retrograde processes of the parenchyma. Sections of the ischiatic nerve in most of our cases were studied, but abnormalities were not observed.

Pathologic Histology of Myeloid Leukosis

We have not accumulated enough material from cases of spontaneous myeloid leukosis to permit a comprehensive study of its histopathology. We have observed the condition repeatedly following its experimental transmission, but are not sure that the histologic picture is entirely comparable in the spontaneous and experimentally induced forms of the disease. We hope to investigate this phase of the problem more completely as additional cases of the spontaneous form of myeloid leukosis are obtainable.

Generally speaking, the nature of myeloid leukosis is such as to lead one to expect significant alterations in the bone-marrow and in the circulating blood. The disease has its inception in the granuloblastic elements of the bone marrow which undergo marked hyperplasia, due to a proliferative overgrowth of these cells. As the condition becomes more severe, the excessive pro-

duction of primitive myeloid cells eventually results in marked invasion of the blood-stream, where a migratory state ensues. The resultant leukemia is frequently profound, and in such organs as the liver and spleen there may be cellular engorgement of the blood-vascular channels, simulating that seen in erythroleukosis. The ever-increasing number of immature myeloid cells are derived from the parent myeloid tissue in the bone-marrow and by mitoses of the cells of the circulating blood in cases of leukosis. In the liver irregular extravascular diffuse groups of myeloblastic cells not uncommonly occur.

The major vessels of the liver usually contain such large numbers of immature myeloid cells as to disclose the true nature of the condition. A study of sectional views of such a vessel, even though the tissue is stained with hematoxylin and eosin, reveals such a striking predominance of unripe myeloblastic cells and a corresponding diminution of erythrocytes as to be characteristic. A comparison of the ratio of erythrocytes to leukocytes readily explains the anemia which usually is so apparent in chickens affected with myeloid leukosis.

DIAGNOSIS

The diagnosis of leukosis and the recognition of its specific form in a given chicken can be accomplished with confidence only by a careful consideration of all available data on a given case. Although study of the blood is essential, it should always be supplemented by a microscopic examination of such tissues as the bone-marrow, liver and spleen.

The presence of anemia in chickens is strongly suggestive of leukosis. On general examination the anemia is manifested by paleness of the wattles, comb and mucous membranes. Whether the anemia is primary or secondary can be determined by the further examination of the blood or by examination at necropsy. If the anemia is due to leukosis, a blood-film appropriately stained will reveal characteristic immature erythroblastic or myeloblastic cells in varying proportions. If the anemia is not associated with leukosis, other factors which may be contributory to the anemic state should be looked for. These include parasitisms, chronic infectious diseases, intoxication and certain neoplasms. A great deal more can be learned about the characteristics of abnormal blood-cells from a smear stained by Wright's method than by examining cells of a similar nature in fixed and embedded tissues stained by such stains as hematoxylin and eosin (fig. 7).

One should recognize the occurrence of some myelocytic and myeloblastic cells in most cases of erythroleukosis and likewise the fact that in the myeloid forms of the disease erythroblastic cells are not uncommon. Since these occur when the disease is predominantly erythroleukosis or myeloid leukosis, it is not difficult to account for cases in which myeloid and erythroblastic elements are present in approximately equal numbers. The occurrence of cases in which the leukosis is due to a combination of cells of different histologic types provides suggestive evidence that the myeloblastic and erythroblastic elements are derived from a parent cell common to both, or the incitant agent of leukosis is capable of provoking a proliferative state in both the myeloid and erythroblastic cells and is not necessarily specific for each.

Since leukosis of chickens may be myeloid or erythroblastic, or a mixture of these forms, the designation in a given case of the specific character of the leukosis is perhaps of more academic than practical significance. The essential point is to recognize the existence of the condition and to be able to distinguish it from lymphocytoma and myelocytoma with which leukosis is confused too commonly.

As a basis for distinguishing leukosis from lymphocytoma and myelocytoma, one must bear in mind that leukosis is primarily a disturbance which makes itself evident in the blood-stream, whereas lymphocytoma and myelocytoma are essentially extravascular processes. The lesions present in the latter conditions are in conformity with this conception. The foci of neoplastic cells in lymphocytoma and myelocytoma tend to occur in large, well-defined masses in the tissues involved, whereas in leukosis the involvement is confined to the vascular bed of the respective tissues. The liver in leukosis occasionally may contain small foci of cells but these differ markedly from the large diffuse or discrete masses seen in lymphocytoma.

The myeloblastic cells in myelocytoma are more mature than those that occur in myeloid leukosis. They may be distinguished as metamyelocytes and myelocytes, whereas in myeloid leukosis the myeloblastic cells which predominate in the blood-stream are leukoblasts and promyelocytes, which cells are devoid of acidophilic granules in the cytoplasm (fig. 5).

The type cell in lymphocytoma is the undifferentiated lymphocyte, which should not be confused with the so-called lymphoid cell seen in the blood of erythroleukosis and which is the hemo-

globin-free progenitor of the erythoblastic cells seen in the latter disease.

Occasionally an atypical form of these respective conditions will occur, and a careful analysis will be required of all lesions as revealed by the gross and microscopic examination of the tissues, including the blood. Particularly may it be difficult to separate from leukosis the diffuse forms of lymphocytoma and myelocytoma. Examination of blood-films and histologic study of the bone-marrow and liver usually will disclose the necessary data properly to distinguish a given case.

We see no reason for confusing leukosis of chickens with a certain neuropathic disturbance which frequently is referred to as "fowl paralysis," range paralysis, or *neurolymphomatosis gallinarum,* of which the principal lesion is a nerve infiltration with lymphocyte-like cells.* Occasionally this condition may occur in chickens concurrently with leukosis, but there is no evidence that so-called fowl paralysis induces the pathologic changes characteristic of myeloid leukosis or erythroleukosis. At present there is no conclusive proof that the etiology of leukosis is related to that of fowl paralysis. In leukosis, cells may sometimes infiltrate the sheaths of the larger nerves. This should not, however, be accepted as proof of the etiologic relationship between leukosis and "fowl paralysis." The extremely cellular state of the blood of fowls affected with leukosis provides a favorable circumstance for extravascular infiltration of all tissues, including nerves. Another explanation for the infiltration of nerves with these cells is the fact that in the various tissues slight hemorrhage not uncommonly occurs from rupture of the smaller blood vessels.

TRANSMISSIBILITY

Although the experimental transmission of leukosis has been placed on a rather secure basis by the work of Furth, Stubbs and Furth and Jármai, the question of whether the disease is communicated spontaneously from affected to healthy chickens has not been settled. There is no evidence that the disease can be transmitted to healthy chickens by their cohabitation with sick

*This view is still maintained, although since this paper was written, a paper by Patterson, Wilcke, Murray and Henderson (Patterson, F. D., Wilcke, H. L., Murray, C., and Henderson, E. W.: So-called range paralysis of the chicken. Jour. A. V. M. A., lxxxi (1932), n. s. 34 (6), pp. 747-767) has appeared, in which the following conclusions in part were drawn: "The manifestation found in the eye, nerves, and central nervous system of cases of so-called range paralysis are expressions of a complex disease process which is by no means confined to the above locations, but may manifest itself in practically any organ or tissue of the chicken. When the bone-marrow and other hematopoietic organs are involved, manifestations of myeloid leukemia and erythroleucosis often occur."

chickens, and it has been shown by Jármai that the sperm of the male is not an instrument of transmission. Jármai and his collaborators attempted to demonstrate without success the development of leukosis in chickens hatched from eggs obtained from affected hens. The same workers likewise failed to transmit the disease to chickens that were hatched from eggs artificially inoculated with infective material before being incubated.

Jármai originally contended that leukosis of chickens could be transmitted by blood-sucking parasites, but more recent investigations have caused him to alter this view. In a recent communication, Jármai and his coworkers described their efforts to transfer the disease from affected to healthy chickens through the agency of the tick, *Argas miniatus*. After starving the parasites for 20 days, they were permitted to engorge themselves by feeding on a chicken affected with leukosis. By emulsifying the engorged ticks with physiologic sodium chlorid solution and injecting the inoculum into healthy chickens, leukosis developed, provided the infective blood had not been contained within the body of the parasite longer than ten days. When the injected parasites were attached to the skin of healthy chickens, the disease did not develop. Jármai mentioned that the chicken-house, in which were both healthy chickens and chickens with leukosis, harbored numerous parasites and yet there was no evidence that the disease was transmitted from one chicken to another. He concluded that although the possibility of an infection exists, the parasites cannot be credited with the rôle of transmission under natural conditions.

The reason for the failure of the parasite to infect healthy chickens probably is due to the fact that the infective agent has lost its potency when a new host is finally attacked. The freshly engorged ticks do not seek new hosts immediately, and the passing of time brings about a diminution in the infectiousness of the inciting agent which eventually becomes impotent. Furthermore, the fact that the bite of the parasite introduces the so-called virus subcutaneously, where it is innocuous in small amounts even if potent by other routes of inoculation, is against the transmission of the disease by these insects.

Jármai also claimed that all natural body secretions of chickens affected with leukosis are devoid of the infective agent, which fact makes the reason for the spontaneous occurrence of several cases in the same flocks difficult to explain on the basis of contagion.

So far attempts to infect artificially species of birds other than the domestic chicken have been unsuccessful. Stubbs and Furth failed to transmit the disease to ten guinea fowls and twelve pigeons. Seldom does leukosis develop in all chickens inoculated and a 50 per cent failure is not uncommon in earlier generations of the transmissible existence of a given strain of the disease. Although the percentage of successes may appear to increase slightly as the number of experimental generations is extended, some chickens may be encountered that are profoundly resistant and cannot be infected regardless of the amount of infective agent inoculated. Furth recorded success in inducing leukosis in healthy chickens by the injection of 0.000,001 cc of infective plasma and the same worker recorded success with a cell suspension of 0.00,001 cc. Much larger doses are usually used in transmitting the disease experimentally.

Experimental transmission can be accounted for on the basis of transplantation in a manner comparable to a tissue graft, or by the introduction of a cell-free filtrate containing the specific inciting factor, which stimulates the hematopoietic elements of the bone-marrow to excessive reproductive activity. Transmission is accomplished best by intravenous injection of whole blood, although emulsions of tissue of chickens dying with the disease usually are potent.

THE BIOLOGY OF THE SUBSTANCE RESPONSIBLE FOR THE EXPERIMENTAL TRANSMISSION OF LEUKOSIS

It is now generally agreed that there exists in most, if not all, chickens affected with leukosis a transmitting agent that is capable of passing through filters. Furth and Miller found that the substance responsible for the transmission of the disease would pass through all types of silicious filters and the ability of the transmitting agent to filter through collodion membranes indicates a substance of extremely small dimensions. Jármai and his collaborators contended that the transmitting substance was filtrable to the degree to which the filtering apparatus permits the passage of the albumin molecule.

Furth studied the tenacity of the infective substance of leukosis and found it to be extremely resistant to drying; it retained its viability for at least 54 days after being desiccated. Preserved in a 50 per cent solution of glycerin, activity was retained for at least 104 days. Freezing blood of chickens affected with leukosis, even by submersion in liquid air, did not render the transmitting agent impotent. Furth found that after reten-

tion in the incubator at 37.5° C. for a period of two weeks the substance was no longer capable of producing the disease. It retained some of its activity after 14 days in the refrigerator at 4° C., but became completely innocuous after 21 days at this temperature.

Attempts definitely to identify the specific substance capable of transmitting leukosis from diseased to healthy chickens have been futile. The exact character of the transmitting factor therefore remains uncertain. Although much has been learned concerning the biology of the agent, but little is known of its physical structure. Since the substance is present in the erythroblastic cells, and to a lesser degree in the plasma of the chickens affected, Jármai and his collaborators are inclined to believe that the exciting factor is not an independent living organism but an enzymatic product of the immature erythrocytes. Whether leukosis should be considered a form of neoplasia, as Jármai believes, is problematic, although there is much to support such a designation. Certainly the disease has no counterpart in comparative pathology, which is indeed unique.

Method of Experimental Transmission

The following technic has been used for the establishing of transmissible strains of leukosis in our laboratory, and for the perpetuation of a strain once established. The affected chicken, or donor, is selected in the later stages of the disease, preferably just before death. Emulsions prepared from the liver and spleen may be suitable if prepared aseptically and used soon after the death of the affected chickens. Whole blood, however, is the most satisfactory tissue for transmission experiments.

An anticoagulant must be used to prevent clotting of the blood in the syringe and needle. Heparin has been utilized in our experiments. By making a solution of 50 mg of heparin in 0.5 cc of Ringer's solution, and leaving this solution in a Luer syringe with a cork thrust onto the needle, the syringe and solution can be sterilized by boiling. Blood is easily obtained by venipuncture from one of the wing veins at the humeroradial articulation. As much blood as desired can be withdrawn, although usually 4 to 5 cc is sufficient. The syringe should be rotated while the blood is being withdrawn to secure a mixture of the heparin. The amount injected into the susceptible chickens may vary up to 0.5 cc, which is the amount usually used by us in the first passage.

Emulsions of liver, spleen or bone-marrow may be made with

blood-serum from healthy chickens, or Ringer's solution may be used as a diluent. The material is put in a sterile mortar and ground, after which the coarser particles may be removed by filtering through gauze. This can be injected intravenously, with little apparent harm to the recipient from the minute particles which remain in the emulsion. Unless the emulsion is filtered or the heavier particles are permitted to settle, its injection may be followed by sudden death.

REFERENCES

[1]Ellermann, V.: Experimentelle Leukämie bei Hühnern. Verhandl. d. deutsch. path. Gesellsch., xii (1908), pp. 224-225.
[2]Idem: Die übertragbare Hühnerleukose. (Julius Springer, Berlin, 1918.)
[3]Idem: Histogenese der übertragbaren Hühnerleukose. IV. Zusammenfassende Betrachtungen. Folia Haematol. Arch., xxix (1923), pp. 203-212.
[4]Hirschfeld, H., and Jacoby, M.: Zur Kenntnis der übertragbaren Hühnerleukämie. Berl. Klin. Wchnschr., i (1909), pp. 159-160.
[5]Schmeisser, H. C.: Spontaneous and experimental leukemia of the fowl. Jour. Exp. Med., xxii (1915), pp. 820-838.
[6]Andersen, C. W., and Bang, O.: La leucémie ou leucose transmissible des poules. (B. Bang, Kopenhagen, 1928, pp. 355-389.) Abst., Cent. f. Bakt., xcii (1929), p. 283.
[7]Furth, J.: Observations with a new transmissible strain of the leucosis (leucemia) of fowls. Jour. Exp. Med., liii (1931), pp. 243-267.
[8]Idem: Erythroleukosis and the anemias of the fowl. Arch. Path., xii (1931), pp. 1-30.
[9]Idem: Studies on the nature of the agent transmitting leucosis to fowls. I. Its concentration in blood cells and plasma and relation to the incubation period. Jour. Exp. Med., lv (1932), pp. 465-478.
[10]Idem: Studies on the nature of the agent transmitting leucosis to fowls. III. Resistance to desiccation, to glycerine, to freezing and thawing; survival at ice box and incubator temperatures. Jour. Exp. Med., lv (1932), pp. 495-504.
[11]Furth, J., and Miller, H. K.: Studies on the nature of the agent transmitting leucosis of fowls. II. Filtration of leucemic plasma. Jour. Exp. Med., lv (1932), pp. 479-493.
[12]Jármai, K.: Beiträge zur Kenntnis der Hühnerleukose. Arch. f. Wiss. prakt. Tier., lxii (1930), pp. 113-131.
[13]Engelbreth-Holm, J.: Bericht über einen neuen Stamm Hühnerleukose. Zeit. f. Immun. u. Exp. Therap., lxxiii (1931), pp. 126-136.
[14]Engelbreth-Holm, J., and Meyer, A. R.; Ueber die uebertragung der Hühnerleukose auf Kücken. Zeit. f. Immun. u. Exp. Therap., lxxiv (1932), pp. 347-352.
[15]Jármai, K., Tibor, S., and Ladislaus, F.: Neuere Beiträge zur Kenntnis der übertragbaren Hühnerleukose. Arch. f. Wiss. prakt. Tier., lxv (1932), pp. 46-71.
[16]Feldman, W. H.: Neoplasms of Domesticated Animals. (W. B. Saunders Co., Philadelphia, 1932.) p. 221.
[17]Mathews, F. P.: Leukochloroma in the common fowl. Arch. Path., vii (1929), pp. 442-457.
[18]Stubbs, E. L., and Furth, J.: Transmission experiments with leucosis of fowls. Jour. Exp. Med., liii (1931), pp. 269-276.
[19]Idem: Relation of age and breed to susceptibility in leucosis of fowls. Proc. Soc. Exp. Biol. & Med., xxviii (1931), pp. 986-987.
[20]Latimer, H. B.: The relative postnatal growth of the systems and organs of the chicken. Anat. Rec., xxxi (1925), pp. 233-253.
[21]Scarborough, R. A.: The blood picture of normal laboratory animals. Yale Jour. Biol. & Med., iv (1931), pp. 199-206.
[22]Wiseman, B. K.: An improved method for obtaining total white cell count in avian blood. Proc. Soc. Exp. Biol. & Med., xxviii (1931), pp. 1030-1033.
[23]Sheard, C., and Sanford, A. H.: A photo-electric hemoglobinometer. Jour. Lab. & Clin. Med., xiv (1929), pp. 558-574.

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CRYPTORCHIDISM AND ITS ECONOMIC IMPORTANCE TO THE PRODUCER OF SWINE AND THE PROCESSOR OF PORK PRODUCTS*

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Cryptorchidism, or the failure of one or both testicles to descend into the normal position in the scrotum, is of common occurrence in swine. Swine-producers, in general, have discriminated severely against the use of herd boars with only one testicle in the scrotum, and have even insisted that, when two testicles are present in the scrotum, they be normal in size as well as prominent in outline. The reason for objecting to the use of cryptorchid boars has been based largely upon suspicions of impaired fertility. The same reason has been advanced, although with less emphasis, for the objection to lack of development in the scrotum even though the testicles are descended normally. Recognized judges of swine at live stock shows are unanimous in their rejection of cryptorchid boars, and they discriminate severely against young boars that show a pronounced lack of development in the scrotum.

PREVALENCE OF CRYPTORCHIDISM

Cryptorchidism occurs in all farm animals, including the dog. Bayer,[1] in a summary of clinical cases, makes note of 1,997 cases of cryptorchidism in the horse, 153 in swine, and seven in cattle, in which left and right cryptorchidism occurred with almost equal frequency in horses and swine. Obviously, cases of this nature are brought to the attention of the practicing veterinarian much more often when they occur in horses than when they occur in other domestic animals, so the figures given may not be representative of the relative frequency with which the defect occurs in these species. Lush, Jones and Dameron[2] found 63 cryptorchid Angora goats in a male population of 1,174. In these cryptorchid Angora goats it was the right testicle which failed to descend. In 77 cases of cryptorchidism in the horse, Hobday[3] found eleven bilateral and 66 unilateral cryptorchids, 36 having the right and 30 the left testicle retained. This defect is found also in sheep, but apparently with less frequency than in horses, cattle, goats or swine. Williams[4] has found it most common in horses and swine. Warwick[5] reports 36 cases of cryptorchidism

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in swine, of which "22.2 per cent were on the right, 69.4 per cent on the left, and 8.3 per cent were bilateral." McKenzie[6] reports five sows that produced ten cryptorchid pigs. The author has under observation a family of dogs involving two unilateral and two bilateral cryptorchids.

Kinsley[7] reports that DeWolf inspected 4,671 male swine and found 35 cryptorchids, of which 28 were single and seven double cryptorchids. Busman, in a count covering a total of 103,000 hogs, observed 264 single and 49 double cryptorchids. Jelen found 2,138 cryptorchids in a total of 534,486, and Shelton found 493 cryptorchids among 142,000 hogs.* (See table I.)

TABLE I—*Summary of numbers and percentages of cryptorchids reported by Busman, DeWolf, Jelen and Shelton.*

INVESTIGATOR	HOGS OBSERVED	MALES	CRYPTORCHIDS	CRYPTORCHIDS ON BASIS OF TOTAL MALES %
Busman (Chicago)..	103,000	49,018*	313	0.64
DeWolf.....		4,671	35	0.79
Jelen (Omaha).....	534,486	254,312	2,138	0.84
Shelton (Denver)...	142,000	67,578	493	0.73
Totals.....	779,486	375,579	2,979	

*The figures for males have been derived, where necessary, by taking 47.59 per cent of the total number of hogs. In the sex classification based upon figures in the U. S. Department of Agriculture Yearbook, 1930, table 384, barrows comprised 47.59 per cent of the total number of hogs from 1923 to 1929 inclusive.

On the basis of these figures, 0.378 per cent of all hogs inspected were cryptorchids, or 37.8 cryptorchids in each 10,000. If this figure is applied to males only, then 0.793 per cent of all males inspected were cryptorchids, or 79.3 cryptorchids in each 10,000. If 37.8 cryptorchids for each 10,000 hogs is applicable to the average annual receipts of swine at public stockyards, it would mean that approximately 170,000 cryptorchids reach the market in the United States each year (U. S. D. A. Yearbook, 1932, table 332.) It appears from these figures that cryptorchidism among swine is rather prevalent.

It will be noted in table I that the findings of these investigators are rather uniform, inasmuch as Busman found 0.64, DeWolf 0.79, Jelen 0.84, and Shelton 0.73 per cent cryptorchids among the populations studied. A possible cause for variation in the prevalence of cryptorchidism in different sections of the

country is pointed out under inheritance of cryptorchidism in this paper.

Figures based upon estimates also have an application in a study of this problem, especially when the estimators have had long experience in the federal meat-inspection service in swine-producing areas other than those from which the information came that is noted in detail above. Aufente* estimates that from 20 to 30 cryptorchids are found in each 10,000 hogs that have been inspected under his supervision, and Brodner* estimates 10 for each 10,000 hogs.

Inheritance of Cryptorchidism

Cryptorchidism is generally considered hereditary. Specific information, however, is not available for all of our domestic animals. Lush, Jones, and Dameron,[2] in working with Angora goats, found on the basis of breeding experiments that cryptorchidism is inherited or at least that many cases of it are, but the exact manner of its inheritance has not yet been discovered in detail. Hadley and Warwick,[8] in discussing defects that are probably inherited, state that "Cryptorchidism is likely heritable in other animals as well as in goats, but this has not been experimentally proved." Kinsley[7] reports that "nearly five per cent of the male progeny of one of Missouri's most famous boars, Chief Tecumseh II, were cryptorchids," and, further, that "Liberty Chief and Chief I know, two boars sired by Chief Tecumseh II, were noted breeders, and from three to five per cent of their get were cryptorchids. Chief Perfection II, sired by Chief Tecumseh II, was the sire of Chief Cherokee Perfection, that in turn sired about three per cent of cryptorchids."

Aufente has found, as a result of eight years of experience in Georgia, Alabama and Florida, where it is customary for great numbers of hogs to run at large, that cryptorchidism exists to a larger percentage than elsewhere because of lack of proper control covering the affected animals, an observation which indicates that the defect is inherited. McPhee, Russel and Zeller[9] have identified the ridgling character as one of a number of recessive factors that appeared in their inbreeding work with swine. These authors report also that they have unpublished

*Information to the author from the following, who are federal inspectors-in-charge at the packing centers indicated: Dr. H. Busman, Union Stock Yards, Chicago; Dr. F. Jelen, Omaha (reporting data from Dr. W. R. Richards, supervisor at one of the local packing plants); Dr. J. R. Aufente, Spokane; Dr. M. Brodner, San Francisco; Dr. J. E. Shelton, Denver. (Dr. J. R. Mohler, Chief, Bureau of Animal Industry, United States Department of Agriculture, has approved the publication of data supplied from the Meat Inspection Service.)

data which strongly indicate that the ridgling character is inherited.

In a population of 107 pigs, both sexes, sired by one boar and out of twelve different sows, McKenzie[6] found ten cryptorchids, which came from seven different litters farrowed by five different sows. The sire of the pigs was not related to the sows, but, of the five sows, four were related to each other, one being the dam of the other three. When the sire of the ten cryptorchid boars was mated to his daughter, a littermate to the cryptorchid boar pig, one sow pig, three normal boar pigs and two cryptorchid boar pigs were farrowed. When a gilt was bred to a littermate cryptorchid boar, one sow pig, two normal and two cryptorchid boar pigs were produced. Since about 50 per cent of the male progeny were cryptorchid, McKenzie concludes that cryptorchidism seems to be inherited.

One of the unilateral dogs previously referred to in this paper is the sire of the other unilateral and the two bilateral cryptorchids that were mentioned in the same connection.

Cause of Cryptorchidism

The cause for the failure of testicles to descend probably has not been fully determined. Williams[4] reports cryptorchidy "due to simple arrest in development." It probably is not adequate to give consideration only to the internal vaginal ring, inguinal canal, and external inguinal ring for the explanation as, no doubt, the attachment of the testicles is of some significance in their descent. Dollar[10] has defined three types of cryptorchidism: "*retentio abdominalis*, when the testicle lies near the upper wall of the abdomen; *retentio iliaca*, when it is near the inner abdominal ring, and *retentio inguinalis*, when it is within the inguinal canal." It is likely that the *retentio abdominalis*, and in some cases the *retentio iliaca* type of cryptorchidism, must be explained, in part at least, by the arrest of development in the attachments of the testicles.

In discussing the descent of the testis, Smith[11] points out that "a pouch of peritoneum grows down into the inguinal canal, carrying with it fibers of the abdominal muscles, which subsequently form the cremaster. Extending from the epididymis of the fetal testicle, to the bottom of what will become the scrotum, is a cord known as the *gubernaculum testis* which, by gradually shortening its length, guides the testicle through the inguinal canal into its final position." Smith further reports that testicles are intra-abdominal during the greater part of

fetal life. This statement is in accord with an investigation by Warwick[5], who found that "eighty-day fetuses had the testicles yet within the abdominal cavity, near but not in the internal vaginal rings, while the gubernacula were descended into the subcutaneous rings but not to the scrota. The 100-day and 110-day fetuses showed testicles in the scrotal sacs along with the bunched up masses of gubernacula." It was also determined on the basis of measurements of the testicles and epididymis, internal vaginal rings, and external inguinal rings of 38 newborn pigs, that in every case the testicle was larger than the internal vaginal ring. However, upon examination of untimed fetuses, whose testicles were just entering the vaginal ring, he found that the size of the ring was only very slightly larger than that of the testicle in each case. It was found further by Warwick that the internal vaginal rings of newborn pigs are slightly larger than those of fetuses with undescended testicles, and he concludes that the vaginal canal does not contract after the descent of the testicles and, that, as a matter of fact, it actually increases in size but at a slower rate than the testicle.

Lush, Jones, and Dameron[2] found the cryptorchid testes in Angora goats usually attached to various parts of the wall of the abdominal cavity or to other organs, such as the intestines, by thin folds or cords of the membranous connective tissue often several inches in length. They conclude that "it seems likely that these attachments are the primary cause of cryptorchidism by preventing the descent of the testis during embryonic life to its normal position in the scrotum."

Castrating Risks

Kinsley[7] reports that "cryptorchidism diminishes the value of a pig because of the greater castrating risk," and Hadley[12] points out that "the operation is more dangerous than ordinary castration, as it usually requires an artificial opening into the abdominal cavity." This exposes the peritoneum to infection and may result in peritonitis from which death is likely to result. On the basis of the data previously presented in this paper, however, it appears that a large number of producers do not remove the cryptorchid testes from an appreciable number of pigs.

Secondary Sexual Characters

Investigators of natural and experimental cryptorchids in many mammalian species are agreed in general that bilateral, and also unilateral, cryptorchids from which the scrotal testicle has been removed, develop secondary sexual characters, and in some cases, as, for instance, in the "rig" or cryptorchid horse, sex-

ual activity often is exaggerated (Crew[13]). There is some variation in the sexual activity among cryptorchids of a species and it is probable that under practical conditions cryptorchidism is of less significance in one species than in another. Cryptorchid horses uniformly develop sexual activity. Horses obviously are allowed to grow to full maturity and have every opportunity to develop this characteristic. Cryptorchid swine, on the other hand, usually go to market at from six to eight months of age, at which time they are very immature. Likewise, cryptorchid

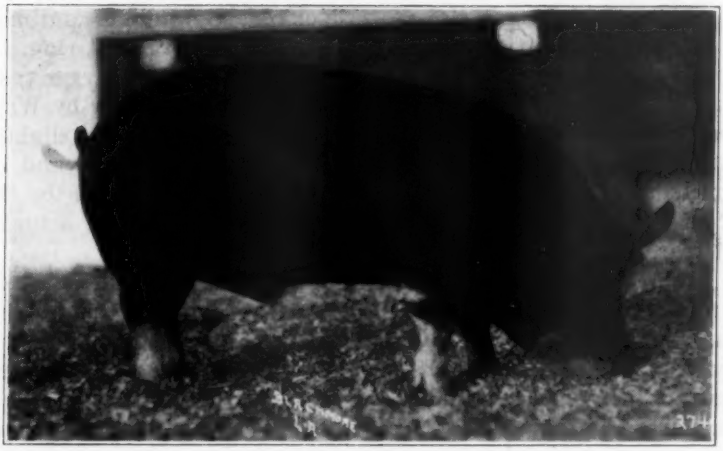

FIG. 1. Cryptorchid pig that did not show sexual development when he was nine months of age. The carcass from this pig did not have sex odor.

ram lambs meet the same fate at an early age. This lack of opportunity to become mature may have some influence on the differences that appear to obtain, which may make cryptorchidism of less practical importance in one species than in another.

During the last four years, the author[14, 15] has made observation of eight natural unilateral cryptorchid boar pigs from which the scrotal testicle was removed when the pigs were from eight to ten weeks old. These cryptorchids were developed in the same lots with gilts and barrows and, with one exception, were marketed at about six months of age at which time they averaged approximately 200 pounds. One of the pigs, the exception just referred to, was exhibited by the owners at a prominent live stock show where he won second in his class at the age of eight months (fig. 1). He was shipped to this station for further observation. He was kept with gilts throughout November—the

breeding season for sows that produce March litters—but showed no sign of sexual activity.

In a survey among seven producers of swine who have had cryptorchid boar pigs in their herds during the last four years, six reported that the cryptorchids "acted and developed as the barrows," and one found two cryptorchids that became "staggy." McKenzie[6] has found that only one out of ten natural cryptorchids failed to develop masculinity, and Wilcox[16] quotes Ellinger to the effect that "sexual excitement appears quickly" in cryptorchid boars. The age at which the cryptorchids developed masculinity is not mentioned by McKenzie.

Odor and Flavor of Meat Produced by Cryptorchids

Dollar[10] points out that "the flesh of cryptorchid swine usually has a repulsive taste." Wilcox[16] reports that "the meat of cryptorchid boars, in which the retained testicles are atrophied, does not possess an odor more disagreeable than that of barrows." Williams[4] has found that "in a boar, since the cryptorchid testicle, as soon as the animal reaches sex maturity, taints the meat beyond endurance for human food, castration is essential," and Hadley[12] says that "the flesh of ridglings and entire male hogs immediately after killing has a decidedly unpleasant odor similar to that emitted by a living boar, and although it gradually diminishes when the carcass is cooled, it again becomes noticeable when the meat is warmed in the process of cooking. The characteristic odor is also accompanied by objectionable taste, which renders the meat unfit for food." Lush, Jones and Dameron[2] report that "the cryptorchid pig often is castrated in order to protect the flavor of the meat."

Busman found that only ten of the 313 cryptorchids previously referred to in this paper gave enough sexual odor to cause condemnation of the carcass, and Jelen condemned 25 out of 2,138 for sexual odor. Aufente says that a condemnation for cryptorchids has not been reported in the last three years, and attributes this to the fact that nearly all hogs slaughtered under his supervision (Spokane) are of a reasonably young age. Shelton estimates that four are condemned for sexual odor out of every ten. Brodner reports that "it has been our experience at one establishment, where approximately 150,000 hogs were slaughtered during the past year, that only one hog carcass of a cryptorchid was condemned for sexual odor." The eight natural cryptorchid pigs observed at this station passed the federal Meat Inspection Service as "free from undesirable odor" and were approved for the pork trade. Histological examination of the

cryptorchid testis in each specimen showed general germinal inactivity.

Experimental Data

In an effort to examine further into the physiological effect of cryptorchidism upon sexual activity, and upon the odor and flavor of the carcasses produced by cryptorchids, seven pigs were made unilateral cryptorchids under aseptic conditions with the assistance of Dr. E. M. Gildow, Idaho Experiment Station Veterinarian. The right testis was removed from all specimens, except X15, at the time the left testis was made cryptorchid (table II). The right testis of X15 was allowed to remain intact for the duration of the test. The test lasted 75 days, during which time the pigs were on full feed. Six of the pigs (X14 to X19, inclusive) were 114 days old at the time of the operation and one pig (X20) was 295 days old. The six young pigs made an average daily gain of 1.55 pounds per day, and X20 gained 1.75 pounds per day for the entire period. The average initial weight of the young pigs was 98 pounds and the initial weight of X20 was 260 pounds. These gains are satisfactory for pigs of these weights while on full feed. Korenchevsky[17, 18] has found that cryptorchid pigs and cryptorchid rats make more rapid development than castrates. No check was made in this test to confirm this conclusion.

Sexual Development

Sexual activity developed and persisted in three of the specimens (X17, X18 and X20). Two of these (X17 and X20), mated with some difficulty. Samples of semen were drawn and examined but no reproductive cells were found.[19] McKenzie[6] found masculinity developed in nine out of ten. It is probable that had the pigs in this test been allowed to develop to a greater age than approximately six months, there might have been a higher percentage of them develop sexually. External evidences of masculinity were not at all evident. Korenchevsky found that cryptorchids looked more hairy than castrates. Specific information on this point was not clearly evident in this test.

Anatomical Development

McKenzie and Korenchevsky have found cryptorchid testes smaller than normally descended testes in swine. An accurate record was made of the weight, and as accurate a record as possible was made of the length and diameter of the testes of all of these specimens, with a view of determining what effect cryptorchidism would have on the growth of testes. On the basis of the figures in table II, if we assume that the right

testis is equal in size to the left testis, then it appears that the left or cryptorchid testis in each of the younger pigs made some growth. In 75 days, the average increase in length was 2.4 cm, in diameter 1.4 cm, and in weight 32 grams. It will be noted further that the right testis of X15, which was allowed to remain intact for the 75-day period, grew much more rapidly than did the cryptorchid testis of the same specimen. The cryptorchid testis of X20, the 10-month pig, however, atrophied materially, becoming hard and tough in texture. Lack of complete surgical success in the operation, although not apparent, may

TABLE II—*Description of normal and cryptorchid testes.*

SPECIMEN	AGE WHEN MADE CRYPTORCHID (DAYS)	LENGTH OF CRYPTORCHID PERIOD (DAYS)	DESCRIPTION OF TESTES					
			LENGTH (CM)		DIAM. (CM)		WEIGHT (GM)	
			RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT
X14	114	75	7.3	8.4	3.2	4.4	113	135
X16	114	75	7.6	8.8	3.8	4.5	105	94
X17	114	75	7.4	9.0	4.2	5.0	100	141
X18	114	75	6.2	11.0	3.2	6.0	93	184
X19	114	75	6.1	9.5	3.5	5.0	103	120
Average			6.9	9.3	3.6	5.0	103	135
Average increase				2.4		1.4		32
X15*	114	75	12.0	10.2	7.0	6.0	418	149
X20	295	75	14.0	9.0	7.2	5.0	453	120

*The right testis of this pig was allowed to remain intact at the time the left testis was made cryptorchid, but was removed with the cryptorchid testis.

have been responsible, in part at least, for the rather rapid atrophy in this case. It appears from these data that growth of the testes is not stopped entirely but that it is arrested materially when they are replaced in the abdominal cavity of pigs less than six months of age, while in older pigs that have attained sexual maturity the tissue seems to atrophy.

The seminal vesicles, prostate and Cowper's glands were materially larger than were those organs of castrates of the same age with which they were compared. This prevailed uniformly in all specimens. It would seem that the development of these sex organs is influenced by testicular hormones that persist in the cryptorchid testes. Korenchevsky has concluded in his work with cryptorchid rats that "hormones stimulating the sexual organs are produced by both the seminiferous and the Leydig's cells."

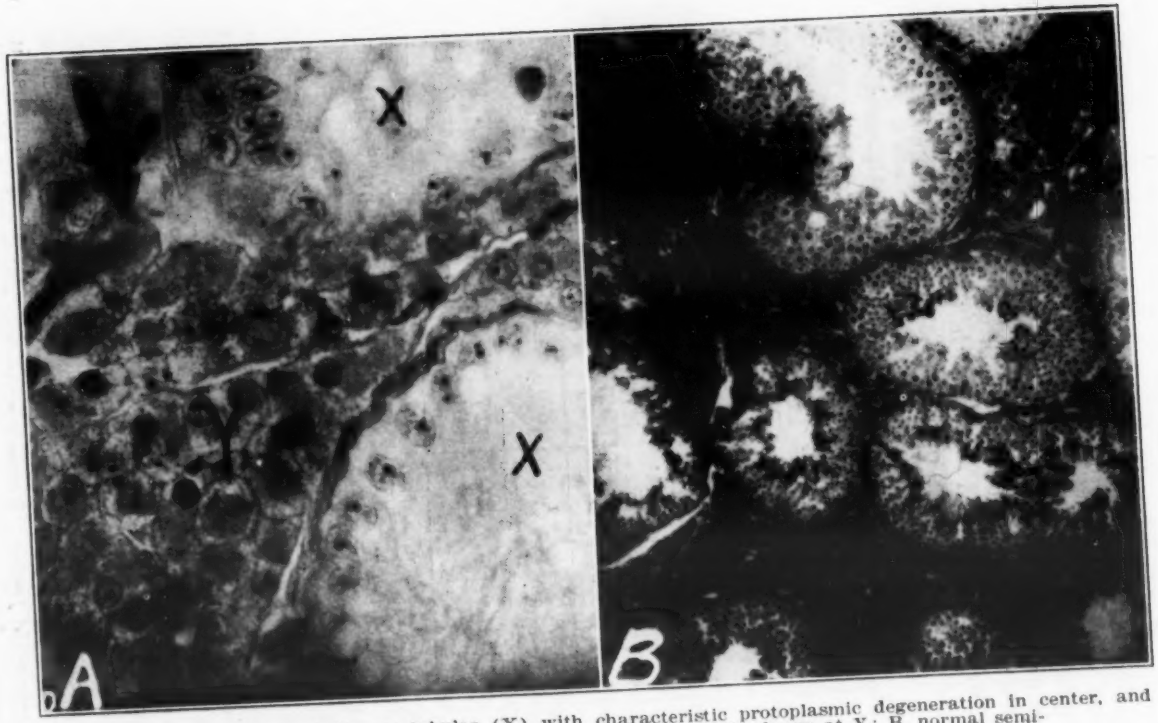

FIG. 2. A, cryptorchid seminiferous tubules (X) with characteristic protoplasmic degeneration in center, and probably largely Sertoli cells at the periphery; Leydig cells shown at Y; B, normal seminiferous tubules showing very active reproductive tissue.

Carcass Tests

At the end of the cryptorchid period, all specimens were slaughtered and the customary federal examination made of the carcasses for odor when they were still warm. The author was assisted by Dr. I. L. Barstow, federal inspector at the Hagan and Cushing packing plant, Moscow, Idaho, in making the "steam pipe" and "hot plate" odor tests of the properly chilled carcasses. In the warm-carcass examination the carcass of X17 was suspected and "retained" for further inspection. All the other carcasses passed the warm-carcass test satisfactorily. All of the carcasses, including X17, passed the inspection satisfactorily in the chilled condition by applying the heat tests mentioned above. While the number of specimens in this test is small, it seems to indicate that there is not so much likelihood that carcasses from cryptorchid pigs that are less than six months of age will be condemned for sexual odor. This conclusion is in agreement with information previously discussed in this paper.

Histological Data

Immediately after each animal was killed, sections were made of the cryptorchid tissue. The tissue was fixed with Bouin's, and stained with Haidenhain's hematoxylin.

In common with cryptorchid tissue in other mammals, concerning which there is much available current literature, the seminiferous tubules were very small and the reproductive tissues very badly atrophied, consisting principally of a layer of cells at the periphery of the tubule. Many of these cells are probably Sertoli's cells. It is extremely difficult to identify with certainty the cells in the cryptorchid seminiferous tubules. Degenerated protoplasm and fat globules are found in the lumen of the tubules, and it seems apparent that cell activity in the seminiferous tissue of cryptorchid pigs is much less, if present at all, than it is in the case of similar tissue, for instance, in the horse.[20]

In the pig cryptorchid tissue there appears to be a decided hyperplasia of Leydig cells. In figure 2, A, is a characteristic condition of Leydig cell development in pig cryptorchid tissue.

Summary

1. Cryptorchidism is prevalent in swine and it appears to be inherited. Cryptorchid boars therefore should not be used. Likewise boars that appear normal but that sire cryptorchid pigs, and sows that produce pigs with this defect should not be used for breeding.

2. The castrating of cryptorchid pigs involves some risk.

3. Not all cryptorchid pigs develop sexual activity if the normal testis has been removed.

4. Apparently only a very small percentage of cryptorchid pigs, from which the normal testes have been removed early in life, develop a sexual odor in the carcass. This appears particularly true when such pigs are marketed at around six months of age.

5. General atrophy characterizes the seminiferous tissue of cryptorchid pigs; hyperplasia is apparent in the Leydig cells, and the Sertoli cells appear more normal.

6. Mature germ cells are absent, and inactivity is typical in the seminiferous tubules.

7. It would seem from the data presented in this paper that the loss to the processor of pork products, and the annoyance to the producer of cryptorchid pigs is not of much economic importance when the normally descended testis is removed early in life and the pig is taken to market when around six or seven months of age. It is probable that the producer would sustain bigger losses by castrating his cryptorchid pigs.

References

[1]Bayer, J.: Operationslehre. (William Braumüller, Vienna and Leipzig, 1910.)

[2]Lush, J. L., Jones, J. M., and Dameron, W. H.: The inheritance of cryptorchidism in goats. Tex. Agr. Exp. Sta. Bul. 407 (1930).

[3]Hobday, F. T. G.: The castration of cryptorchid horses and the ovariotomy of troublesome mares. (William R. Jenkins, New York, 1930.)

[4]Williams, W. L.: The Diseases of the Genital Organs of Domestic Animals. (Publ. by the author, Ithaca, N. Y., 1921.)

[5]Warwick, B. L.: A study of hernia in swine. Wis. Agr. Exp. Sta. Res. Bul. 69 (1926).

[6]McKenzie, E. F.: Cryptorchidism in swine, and anatomy of cryptorchid boars. Mo. Agr. Exp. Sta. Bul. 300 (1931).

[7]Kinsley, A. T.: Swine Practice. (American Veterinary Publishing Co., Chicago, 1921.) p. 196.

[8]Hadley, F. B., and Warwick, B. L.: Inherited defects of live stock. Jour. A. V. M. A., lxx (1927), n. s. 23 (4), pp. 492-504.

[9]McPhee, H. G., Russel, E. Z., and Zeller, J.: An inbreeding experiment with Poland China swine. Jour. Hered., xxii (1931), 12, pp. 393-403.

[10]Dollar, J. A. W.: Regional Veterinary Surgery and Operative Technique. (William R. Jenkins, New York, 1912.)

[11]Smith, F.: A Manual of Veterinary Physiology. (Baillière, Tindall & Cox, London, 1921.)

[12]Hadley, F. B.: Principles of Veterinary Science. (W. B. Saunders Co., Philadelphia, 1924.)

[13]Crew, F. A. E.: A histological study of the undescended testes of the horse. Jour. Comp. Path. & Therap., xxxv (1922), pp. 62-69.

[14]Nordby, J. E.: An. Rpt. Idaho Agr. Exp. Sta., Bul. 179 (1930).

[15]Nordby, J. E., and Bollen, W. B.: Temporary impotency in a boar. A preliminary study on motility and pH in fresh semen samples. Corn. Vet., xx (1930), 4, pp. 381-385.

[16]Wilcox, E. V.: Handbook of Meat Inspection. (American Veterinary Publishing Co., Chicago, 1919.)

[17]Korenchevsky, V.: Experimental cryptorchidism in pigs. Jour. Path. & Bact., xxxiii (1930), 3, pp. 683-687.

[18]Idem: The influence of cryptorchidism and of castration on bodyweight, fat deposition, the sexual and endocrine organs of the male rat. Jour. Path. & Bact., xxxiii (1930), 3, pp. 607-636.

[19]Nordby, J. E.: Sterility in boar responsible for reduced size of litters. Jour. A. V. M. A., lxxiv (1929), n. s. 27 (6), pp. 911-914.

[20]Idem: Spermatogonium and spermatocyte degeneration in cryptorchid testis of the horse. Trans. Amer. Microscopical Soc., xlvii (1928), 1, pp. 54-67.

A METHOD FOR THE PREPARATION OF ANTIGEN FOR THE RAPID AGGLUTINATION TEST FOR BANG'S DISEASE WITH A TECHNIC FOR CONDUCTING SUCH TEST*

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FOREWORD

The literature shows that the sensitivity of rapid-test *Bact. abortus* agglutination antigen is standardized by comparing the results with those obtained by the test-tube method. We have directed attention to objections to this method of standardization in three previous publications.[1-3] Anyone who has used the various rapid-test agglutination antigens must realize that they have not been uniform in sensitivity. Uniformity of rapid-test antigen is highly desirable and in fact is essential for the most successful application of this method of diagnosis of Bang's disease.

We are submitting a technic for the preparation of rapid-test *Bact. abortus* agglutination antigen which, within itself, constitutes a standard for this test and is not in any way dependent on the tube test. This technic and standard for the rapid test will give results that closely approximate those obtained by the test-tube method in accordance with the standard adopted by the United States Live Stock Sanitary Association.[4] The agreement will not be perfect. The data in a previous publication[3] teach us that it is not possible, with our present knowledge, to obtain complete agreement between the two tests (rapid and test-tube) with all serums.

It should be understood clearly that we do not feel that the technic described herein is perfect nor as near perfect as it is possible to make it. It is being submitted at this time because we believe that it should result in an improvement, from the standpoint of uniformity, in rapid-test *Bact. abortus* agglutination antigens. This technic, of course, is subject to revision when better methods are available.

PREPARATION OF ANTIGEN

Cultures: Polyvalent antigens are preferred for the reason

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that it has been shown[5] that the antigenic properties of different strains may vary and result in significant differences in sensitivity of antigens. Cultures of at least six typical strains of *Bact. abortus* that show no tendency to spontaneous agglutination and manifest good agglutinability should be used in the preparation of antigen. Strains selected should be cultivated and studied under usual laboratory conditions to determine that they do not contain dissociated forms.

Growth of bacteria: The culture medium selected should be such that the strains will grow well on it. There is very little preference among the media ordinarily employed, namely, nutrient agar containing glycerin, serum-infusion agar, liver-infusion agar, or potato-agar. The cultures should be incubated only until maximum growth is obtained, which is usually from 48 to 72 hours at 37.5° C. The bacteria may be grown either in the atmosphere or in a modified atmosphere containing 10 per cent CO_2. Glass test-tubes, Blake or Erlenmeyer flasks, or flat bottles may be used as containers for the culture medium on which the bacteria are grown.

Removal of bacteria from culture medium: Bacteria should be removed from the culture medium with a solution containing 0.85 per cent C. P. sodium chlorid and 0.5 per cent of melted, loose C. P. phenol crystals in distilled water. Bacteria may be loosened from the medium with a blunt instrument or soaked loose by permitting the phenolized salt solution to stand on the culture for two to three hours. It is convenient to keep the bacterial suspension quite concentrated in removing it from the culture medium. If glass tubes are used for the culture medium, this can be accomplished by washing the bacteria (with the aid of a blunt instrument) from a number of tubes in the same phenolized salt solution by pouring it from one to another. Re-washing the tubes of culture medium is usually advisable to harvest all the organisms. If flasks or bottles are used for the culture medium, relatively small quantities of salt solution should be used and the bacteria soaked loose from the culture medium. The concentrated suspension of bacteria should be carefully filtered through cotton, or cotton, spun glass and gauze.

Heating the bacterial suspension: The bacteria are killed by slow boiling over a flame for ten minutes. The boiling process should be done in a covered glass container to minimize the coagulum formation on the surface of the suspension. Boiling in an open beaker with constant stirring of the bacterial suspension also is a satisfactory technic for minimizing the coagulum forma-

tion. The coagulum will be removed in the subsequent filtration if its formation is not prevented. Its removal is not objectionable except that it is wasteful and reduces the volume of bacteria for antigen production by approximately one-third. After the boiling process, the bacterial suspension is cooled and again filtered as above.

Washing the bacteria: The suspension of killed bacteria is placed in suitable centrifuge-tubes and centrifugalized until the organisms are completely thrown down. Usually this will be accomplished after about two and one-half hours at 2,600 revolutions per minute. The supernatant phenolized salt solution is decanted off.

Resuspending the bacteria: The centrifugalized bacteria are resuspended in an 0.85 per cent solution of C. P. sodium chlorid containing 0.4 per cent gelatin. This solution should be prepared by adding an appropriate quantity of dry gelatin (De-Ashed Eastman Purified Gelatin) to the salt solution and subsequently sterilizing the solution in the autoclave at 15 pounds pressure for 20 minutes. The volume should be returned to its original after sterilization by the addition of sterile distilled water. A sufficient quantity of melted, loose phenol crystals is added to make a 0.5 per cent solution by volume. It is usually advisable to prepare fresh gelatin solutions each time antigen is made. The bacterial suspension should be kept quite concentrated, since in standardizing the bacterial concentration of the antigen, it is easier to dilute the suspension further than it is to concentrate it. After the bacteria have been resuspended, it is advisable to shake vigorously (power machine preferred) to insure a uniform distribution of the bacteria.

Standardization of the bacterial concentration: The bacterial concentration is adjusted to between 10 and 12 per cent by volume by the following technic. Capillary centrifuge-tubes* (fig. 1)[6] are employed for estimating the concentration of bacteria

FIG. 1. Capillary centrifuge-tube.

*Manufactured by Machlett & Son, 50 Williams Street, Long Island City, New York. These tubes can be cleaned conveniently by inserting a long hypodermic needle into the capillary portion of the tube. This needle should be connected with a water faucet with suitable rubber tubing. In this manner tubes can be flushed out conveniently with water, which in turn is followed by alcohol and ether. They are then dried easily.

in the suspension. Place 0.2 cc of the concentrated bacterial suspension in each of at least two centrifuge-tubes. Subsequently the centrifuge-tubes are filled up to the 10-cc mark with physiologic salt solution. The tubes are corked and placed in a centrifuge. The metal tubes in which the glass tubes are placed must have suitable rubber pads in the bottom, and the space around the glass tube should be filled with water or physiologic salt solution. Be sure that the corks will not touch the centrifuge-head when it is in motion. These precautions are necessary to prevent breaking the capillary end of the glass centrifuge-tubes. When these precautions are observed the breakage is negligible. The tubes then are centrifugalized at 2,600 to 2,700 revolutions per minute for 75 minutes.

The capillary portion of the centrifuge-tube is graduated in 0.002 cc, with a maximum reading of 0.05 cc. The amount of bacteria shown in the capillary tube represents the quantity of bacteria in 0.2 cc of the concentrated suspension. If the bacterial concentration of the suspension is between 10 and 12 per cent, the centrifuge-tube reading will be between 0.02 and 0.024 cc. This is the concentration desired and nothing further needs to be done. If the volume of bacteria in the capillary tube is greater or less than these limits (0.02 to 0.024 cc) it is necessary to adjust the bacterial concentration further. The necessary adjustment can be estimated on the basis of the original centrifuge-tube determination. For example, if the centrifuge-tube reading is 0.04 cc, it means that the suspension is approximately 20 per cent bacteria and therefore needs to be diluted nearly 50 per cent. Or, if the reading is only 0.01 cc, it shows that the suspension is only approximately 5 per cent bacteria and will have to be concentrated by centrifugalization and removal of part of the supernatant fluid. After such adjustment, it is advisable again to determine the bacterial concentration as above. This should be done rather than merely to accept the adjustment of the bacterial concentration on the basis of the first centrifuge-tube determination. This procedure reduces the error of this method.

Standardization of pH: It is usually not necessary to alter the pH concentration of the antigen. Sensitivity of antigens having a comparatively broad zone of pH concentration is unaltered.[5]

Dyes as preservatives: Huddleson[7] recommended that dyes be used in the preservation of agglutination antigens for the rapid test. Gentian violet and brilliant green are added, from a 1 per cent aqueous stock solution, so that the final dilution of the

former dye in the bacterial suspension is 1:50,000 and of the latter 1:25,000. The antigen is now ready for use. It is advisable to keep it in a cool dark place in a tightly stoppered container, when not in use.

THE TEST

Serum samples: Suitable instructions for collecting, identifying, preparing and handling blood samples for the agglutination test for Bang's disease have been printed so often that it is not considered necessary to repeat them here.[4]

Preparing the serum-antigen dilutions: Both the serum and the antigen should be brought to room temperature before the test is started. The antigen should be mixed thoroughly by shaking before beginning the test and at frequent intervals during its use. It is convenient and advisable to use five dilutions of serum in antigen for each test. The serum amounts recommended are 0.08, 0.04, 0.02, 0.01 and 0.005 cc. These amounts of serum are delivered on the glass plate with a suitable serological pipette. A clean pipette may be used for each serum or a single pipette may be used, providing it is rinsed thoroughly with 0.5 per cent phenolized physiologic salt solution, after delivering each serum.

Approximately 0.03 cc of antigen then should be placed on each amount of serum. This may be delivered on the glass plate with a dropper pipette or with a serological pipette of the same type as that used for delivering the serum. If the dropper pipette is used, it should be one that delivers approximately 0.03 cc of antigen. Instructions for preparing such standardized dropper pipettes are given by Huddleson and Abell.[8] Care should be taken to hold the dropper pipette in a vertical position to insure accuracy and uniformity in the size of drops delivered. It is convenient to prepare about six different tests at one time. This prevents excessive evaporation of the smaller amounts of serum before the antigen is mixed with it. Slight evaporation of the serum, however, is of no particular importance. The lights in the test-box should not be turned on except during observation of the tests. This prevents excessive warming of the glass plate. Warming the glass plate above room temperature is not only not necessary but is undesirable, because it tends to increase the rate of evaporation of the test materials. In tests of some serums that are very slow to agglutinate the antigen, the test materials may be evaporated on a warm glass plate

before the reaction has had time to occur. The serum and antigen should be mixed thoroughly. This can be accomplished conveniently with an ordinary wooden toothpick, using a separate one for each serum. The dilution containing the smallest amount of serum should be mixed first and then the other dilutions in the order of the increasing amounts of serum. The mixtures should be spread over areas ranging from about three-quarters of an inch in diameter for the smallest amount to approximately one and one-quarter inches for the largest amount of serum. This practice is desirable because the depth of the serum-antigen mixture affects the visibility of agglutination.

When the glass plate has been filled with tests, it should be tilted and rotated slowly and gently four or five times to mix the test materials further. This process also tends to speed up the agglutination reaction.

Observation of tests: The tests should be allowed to stand for at least eight minutes before final observation, unless complete agglutination in all dilutions occurs prior to this time. The time before final observation can well be considerably longer than eight minutes, providing perceptible evaporation of the test materials is prevented. The readings can be made accurately as much as one hour or longer after the tests have been prepared. It is far better to delay final observation for ten to 15 minutes than it is to be too hasty and make readings in less than eight minutes.

When ready to observe the tests, the glass plate should be tilted and rotated gently two or three times. This tends to cause certain slight changes in the appearance of the tests to disappear, that are apparently due to settling of the antigen and not to specific agglutination. The lights in the test-box now are turned on, the glass plate tilted so that the serum-antigen mixtures flow toward the lower edges and the observations made. This technic aids one in seeing the reaction in tests that show only incomplete agglutination.

The agglutination results in individual dilutions should be recorded as negative (—), incomplete (I), or positive (+). Slight traces of agglutination should be ignored and the result recorded as negative. The basis for this policy is given in a previous publication.[3]

The test-box: The accompanying photographs (figs. 2 and 3) illustrate a suitable type of test-box. Blue-print drawings of this test-box are available. The glass cover is an essential

feature. This prevents rapid evaporation of the test materials on the glass plate due to air currents in the room. A series of such boxes or a suitably constructed table, built in sections, is convenient where large numbers of tests are being conducted. The worker can prepare a series of 20 or more tests and allow them to stand for several minutes, then observe and record the results, beginning with the first tests that were set. Thus the testing becomes a continuous operation and there is no waste of time. Identification of the tests can be accomplished easily by using a wax pencil or other simple method of numbering each test.

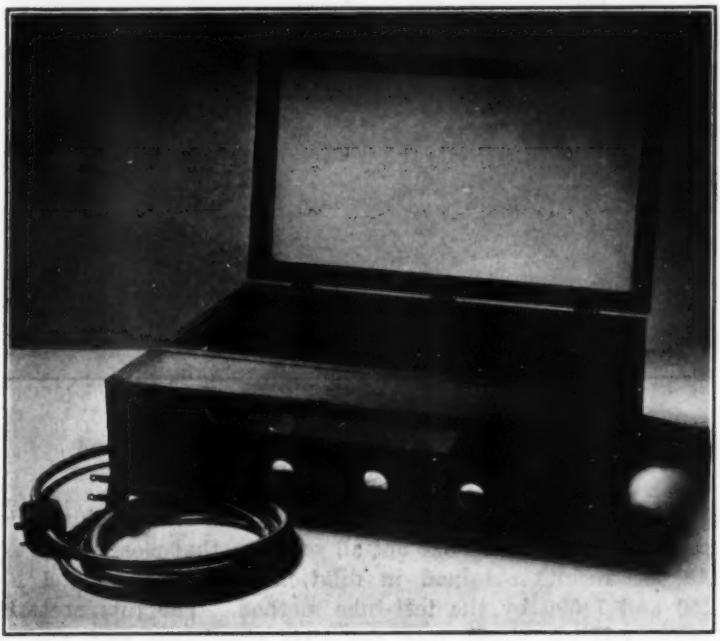

FIG. 2. Test-box open.

Cleaning the glass plate: It is not necessary to employ any elaborate or expensive technic for cleaning the glass plate. The essential thing is to clean it and this is not difficult. A suitable method is to rinse it with ordinary tap water, then rub the surface with a wet, soft sponge and a little sand soap or ordinary cleaning powder, rinse again and rub dry with an ordinary clean towel.

Cleaning and sterilizing serological pipettes: If a separate pipette is used for each serum, it is necessary to force the excess of serum out of the pipette after it has been used and then submerge it in cold water. This prevents the excess serum from drying and clogging the capillary opening in the pipette. If an autoclave is not available, the pipettes may be sterilized by boiling for 15 minutes in an open container. Subsequently they should be stood on end in a warm place (*e. g.*, over a radiator) to drain and dry before being used again.

FIG. 3. Test-box closed.

INTERPRETATION OF RESULTS

The amounts of serum recommended will give agglutination titres (in tests of most but not all serums) that closely approximate the results obtained in dilutions of 1:25, 1:50, 1:100, 1:250 and 1:500 by the test-tube method. The interpretation of agglutination titres into negative, suspicious and positive diagnoses is not uniform in all states. The veterinarian should consult the recommendations on this point that are in vogue in his state. He should comply with the policy of the state of destination in testing for interstate movement of animals. The standard technic for the test-tube method, adopted by the United States Live Stock Sanitary Association, includes recommendations for interpretation of results, as shown in table I. Further discussion of the interpretation of results has been given in a previous publication.[2]

TABLE I—*Interpretation of reactions.*

DILUTIONS				DIAGNOSIS
1:25	1:50	1:100	1:200 or 1:250	
−	−	−	−	Negative
I	−	−	−	Negative
+	−	−	−	Suspicious
+	I	−	−	Suspicious
+	+	−	−	Suspicious
+	+	I	−	Suspicious
+	+	+ and above	−	Positive
				Positive

REFERENCES

[1]Fitch, C. P., and Donham, C. R.: A discussion of some fundamental principles and practices underlying the application of the agglutination tests for Bang's disease. Jour. A. V. M. A., lxxxii (1933), n. s. 35 (1), pp. 46-53.

[2]Donham, C. R.: Some of the things we should recognize concerning the agglutination test for Bang's disease. Jour A. V. M. A., lxxxii (1933), n. s. 35 (3), pp. 327-335.

[3]Donham, C. R., and Fitch, C. P.: Agglutination test in the diagnosis of infectious abortion in cattle (Bang's disease) with special reference to the rapid-test method. (In process of publication.)

[4]Report of 1931 Committee on Bang's Disease (Part I) of the United States Live Stock Sanitary Association. Jour A. V. M. A., lxxx (1932), n. s. 33 (3), pp. 323-325.

[5]Unpublished data.

[6]Fitch, C. P., Donham, C. R., Bishop, L. M., and Boyd, W. L.: Studies of the test tube agglutination test for the diagnosis of Bang's disease (contagious abortion). Minn. Agr. Exp. Sta. Tech. Bul. 73 (1930).

[7]Huddleson, I. F.: The diagnosis of Brucella infection in animals and man by rapid macroscopic agglutination. Mich. State Coll. Tech. Bul. 123 (1932).

[8]Huddleson, I. F., and Abell, E.: Rapid macroscopic agglutination for the serum diagnosis of Bang's abortion disease. Jour. Inf. Dis., xlii (1928), pp. 242-247.

Massachusetts Changes Annual Meeting Date

The Massachusetts Veterinary Association, at the annual meeting held in Boston, April 26, 1933, amended the by-laws of the organization, to provide for holding the annual meeting in January instead of April, as heretofore. The Association year now is the same as the calendar year. The change was brought about as the result of affiliation with the A. V. M. A. Massachusetts is the second state to report this action. Michigan was the first.

More Cattle on Feed in April

A ten per cent increase in the number of cattle on feed in eleven Corn Belt states on April 1, 1933, over the corresponding date last year, is estimated by the U. S. Department of Agriculture. The increase amounts to approximately 130,000 head.

A CHRONIC CARRIER OF FOWL TYPHOID OF TURKEYS*

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Fowl typhoid of turkeys is of considerable economic importance in certain turkey-growing areas and has been reported recently by one of the writers,[1] from California, and by Martinaglia,[2] from South Africa. As far as can be determined, however, there is no published record of chronic ovaritis of turkeys caused by *Salmonella gallinarum*. This report is a description of such a case and is presented for the purpose of suggesting the possibility of transmission of the disease by means of eggs laid by turkey hens that have survived an acute outbreak.

Subject: A Bourbon Red turkey hen T-81, age 2 years.

History: The subject originated in a flock of 300 Bourbon Red turkeys which suffered a 50 per cent mortality in an outbreak of fowl typhoid lasting from September, 1930, to February, 1931.

The disease was first diagnosed in this flock on October 2. On October 8, the survivors, with the exception of 17 controls, were each given a subcutaneous inoculation of 3 cc of an autogenous bacterin. These inoculations were repeated on October 21, January 5 and January 12. The flock was kept under observation during the entire course of the disease. The birds were legbanded and individual notes were made whenever it was deemed desirable. Temperatures were recorded at each time of inoculation of bacterins, blood samples were taken at intervals, and autopsies were made on all the birds that died during the period.

Bird T-81 was in the inoculated group and was not observed to be ill until January 5, 1931. On October 8, however, its temperature was 107.2° F., but by October 21, this had subsided to 106.4. Its temperature on January 5 was 108.8 and typical symptoms of fowl typhoid were noted. Within two days, it had

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improved and its temperature was 107.0. By January 12, it was again normal, with a temperature of 105.8. What effect the third dose of bacterin, given on January 5, had on its rapid recovery is not known, but no evidence of the curative effect of the bacterin was observed in the remainder of the flock.

No record of bird T-81 was kept from January 12 to November 9, 1931, but according to the owner, it remained normal during that period. Just previous to November 9, it and eleven other survivors of the 1930 outbreak were sold to a turkey-grower for breeding purposes. These birds were tested for the presence of *S. gallinarum* agglutinins on November 9 and again on November 28 and bird T-81 alone reacted. The only evidence that any of the others were at any time ill with fowl typhoid consisted of slightly elevated temperatures recorded in two of them during the 1930 outbreak.

The subject was purchased in April, 1932 and placed with a normal male Bronze turkey in a cleaned and disinfected pen. Seventeen eggs were laid from April 14 to June 22, 1932. These were incubated at 37° C. from one to two weeks, after which the entire contents of each were seeded into meat extract broth. After incubation for enrichment purposes, agar plates were inoculated and incubated. In no case was an organism resembling *S. gallinarum* isolated.

On June 3, bird T-81 was still a reactor. The results of the various agglutination tests made with blood from this bird are given in table I. Where available, the temperatures also are recorded.

TABLE I—*Agglutination test results and temperature readings.**

DATE	AGGLUTINATION TEST DILUTIONS					TEMPERATURES	REMARKS
	20	40	80	160	320		
12–20–30	2	0	0	0	0	107.2	Two months after second dose of bacterin
1– 5–31	2	1	0	0	0	108.8	Third dose of bacterin
1–12–31	4	0	0	0	0	105.8	Fourth dose of bacterin
11– 9–31	4	4	3	0	0	Not taken	
11–28–31	4	4	3	0	0	Not taken	
6– 3–32	4	4	1	0	0	Not taken	Serum slightly cloudy

0=no agglutination.

1-4=relative clearing and agglutination.

*Readings made after 24 hours incubation at 37° C. and 24 hours at room temperature.

The inoculations with bacterins probably influenced the tests made previous to January 12. The majority of the other birds in the flock, however, were non-reactors on December 21, which was two months after the second inoculation of the bacterin, and again January 5, the day of the third inoculation. On January 12, only one of the controls (uninoculated) was a reactor, while most of those inoculated on January 5 had titres of 1:80 to 1:320, with a few as high as 1:1280.

Autopsy results: The turkey was noted to be listless a day or so previous to death. It died during the night of July 17, 1932, and was found the following morning. Some postmortem decomposition had taken place. It was in excellent flesh. There was a marked ovaritis, with peritonitis, salpingitis and intestinal adhesions. The latter probably were caused by ruptured ova, which were present in the abdominal cavity. There was one fully developed egg in the oviduct. The ovary contained few normal ova. The diseased ova (fig. 1) varied in size from that of a pea to that of a walnut, and were typical of those seen in pullorum disease of the ovary in chickens.

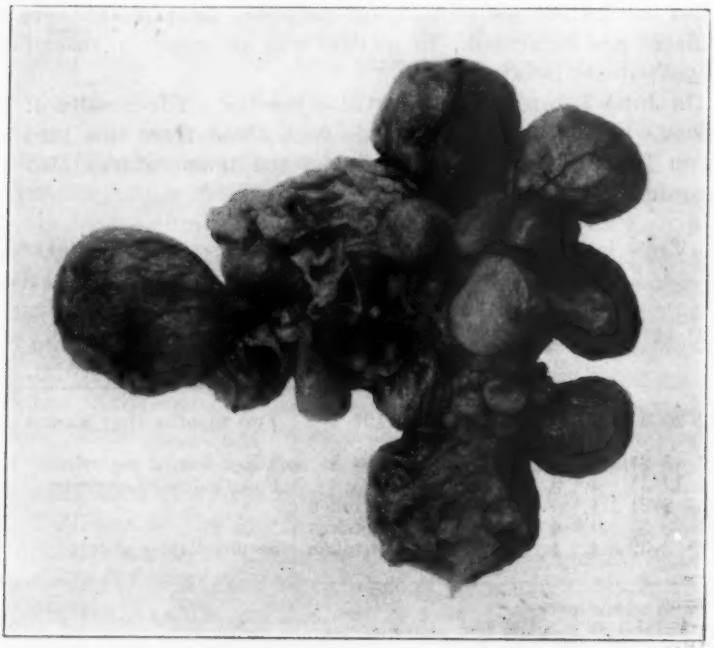

FIG. 1. Ovary from turkey hen T-81, showing diseased ova.

Bacteriological results: Organisms resembling *Pseudomonas pyocyaneus* and *Escherichia coli* were isolated from the liver and one resembling *E. coli* from the oviduct. After preliminary studies, these cultures were discarded. Similar studies on a culture isolated from the ovary indicated that it was probably *S. gallinarum*. It was saved for further study and given the designation M-2. This organism was found to be a Gram-negative, non-motile rod occurring mostly in singles but occasionally in pairs. Its average size, when grown on plain meat extract agar for 48 hours, was 0.5 x 1.0 microns, with extremes of 0.4 to 0.9 x 0.7 to 1.5 microns. Growth was abundant on most media; moderate uniform turbidity was produced in meat extract broth and gelatin was not liquified. Slight acidity induced in litmus milk changed to slight alkalinity after four days, with reduction of the litmus in the lower one-fourth of the tube in 14 days. Complete reduction did not take place. There was partial peptonization in plain milk after 28 days. Nitrates were reduced. H_2S was produced in lead acetate medium.

Acid without gas production was noted in the following carbohydrate media; dextrose, galactose, levulose, maltose, arabinose, xylose, dextrin, glycerol, dulcite and mannose. Glycerol media never became more than faintly acid during the 30 days the cultures were under observation. Maltose, dextrin and dulcite remained faintly acid for several days but finally became markedly acid.

Lactose was not attacked; sucrose became faintly acid for 72 hours, then neutral; inulin became distinctly alkaline in 96 hours; and salicin became alkaline in 120 hours, but became neutral again by the 13th day. A synthetic medium, suggested by Scott,[3] with brom thymol blue as an indicator, was used for all of the carbohydrate fermentation studies.

Two nearly mature rabbits were inoculated intravenously with 0.5 and 1.0 cc respectively, of a 48-hour broth culture, and observed for three weeks. Both became ill and were emaciated after a few days. The titre of each of their bloods at the end of three weeks was 1:640 with antigen prepared from tested stock cultures of *S. gallinarum*. The one receiving 1.0 cc of culture was killed and a pure culture of the causative organism isolated from an abscess in the liver. The other one was kept for an additional month for the purpose of being hyperimmunized with bacterin. It recovered and was normal at the time of autopsy. The culture obtained from the liver abscess in the one rabbit

proved pathogenic for a half-grown rabbit, which died in six days after receiving 1.0 cc of a 48-hour broth culture.

All of the above cultural and pathogenicity tests were paralleled with three strains of *S. gallinarum*, two of which originated from acute outbreaks in turkeys and the third from an acute outbreak in chickens. Cross-agglutinations in equally high dilutions to the homologous antigens in antisera of known and tested strains were obtained. The results with minor variations were alike in all instances. Therefore, the culture M-2 was classified as *S. gallinarum*.

COMMENT

The fact that *S. gallinarum* was isolated from the subject of this case report is evidence that fowl typhoid may be perpetuated from year to year in turkey flocks by means of chronic carriers. That such carriers may transmit the disease in eggs has been suggested, but not proven by the studies made on this bird, since the 17 eggs cultured did not yield the organism. In the instance of pullorum disease carriers, however, it has been proven that the degree of disease in the ovary is not a criterion of the percentage of infected eggs laid. One can not conclude that fowl typhoid in turkeys is transmitted through the egg, until the organism has been isolated from eggs laid by carriers, and from poults that have hatched from such eggs. These results were comparable to those of Beaudette[4] and Beach and Davis,[5] who cultured eggs from chicken carrier hens but failed to isolate *S. gallinarum* in a single instance.

SUMMARY

A case of ovaritis in a Bourbon Red turkey caused by *S. gallinarum* is reported. This bird recovered from an acute case of fowl typhoid during the fall of 1930; and reacted to the agglutination test until its death in July, 1932, when *S. gallinarum* was isolated from its ovary. This diagnosis furnishes evidence that fowl typhoid may be transmitted through eggs of turkey carrier hens, but as no cultures of the organism were obtained from the 17 eggs laid by the bird, positive proof was not secured.

REFERENCES

[1]Hinshaw, W. R.: Fowl typhoid of turkeys. Vet Med., xxv (1930), 12, pp. 514-517.

[2]Martinaglia, G.: A note on *Salmonella gallinarum* infection of ten-day-old chicks and adult turkeys. Jour. So. African Vet Med. Asso., i (1929), 3, pp. 35-36.

[3]Scott, J. P.: A simple mineral agar for the cultivation of Pasteurella boviseptica. Jour. Bact., xx (1930), 1, pp. 9-14.

[4]Beaudette, F. R.: The possible transmission of fowl typhoid through the egg. Jour. A. V. M. A., lxvii (1925), n. s. 20 (6), pp. 741-745.

[5]Beach, J. R., and Davis, D. E.: Acute infection of chicks and chronic infection of the ovaries of hens caused by the fowl typhoid organism. Hilgardia, ii (1927), 12, pp. 411-424.

A COMPLICATED CASE OF PARTURIENT PARESIS*

By J. F. BULLARD, *LaFayette, Ind.*
Purdue University Agricultural Experiment Station
Department of Veterinary Science

This case was thought to be of sufficient interest to report, due to the fact that the acute tympanitic condition masked the symptoms of parturient paresis so completely that the latter condition was not recognized until the tympanites was relieved.

This Jersey cow had no history of parturient paresis during the several previous parturitions.

At 6:15 p. m., the day following parturition, the writer was called. At this time the cow was very badly bloated and in an exhausted and nervous condition. Respirations were very rapid, as well as the pulse. The tongue was protruded and visible mucous membranes showed evidence of early cyanosis.

A mouth speculum was immediately applied and the stomach tube was passed. A large quantity of foul-smelling gas was expelled and the tympany was relieved. Within a very few minutes the respirations were nearly normal and the pulse also had slowed down considerably. A rectal enema was given at this time, which removed a large quantity of hard, dry fecal matter.

As soon as the tympany and associated symptoms subsided, the cow then showed the typical syndrome of milk fever. Two hundred and fifty cc of 20 per cent calcium gluconate solution was injected intravenously. Five minutes after the completion of this injection, the cow appeared brighter and started to ruminate. She was seen by the night man at midnight and was still down, but in a different part of the stall. At 3 a. m. she was up and eating. There was no recurrence of either condition.

ENTROPION OPERATION IN FOALS†

By J. F. BULLARD, *LaFayette, Ind.*
Purdue University Agricultural Experiment Station
Department of Veterinary Science

This report describes a modified operation for entropion, which is much more quickly and easily performed than the regular operation, as described in the standard text-books on surgery.

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†Received for publication, March 20, 1933.

The usual preliminary procedures, such as skin disinfection and so forth, are applied. The operation itself simply consists of inserting a mattress suture through the skin only, on an imaginary line running about one-half inch below the edge of the lower lid and parallel to it. The amount of skin included in the suture is about a quarter of an inch in each direction. (With this suture there will be four puncture holes made by the needle, two entering the skin and two leaving it. These puncture holes roughly form a square, which is about one-quarter inch on each side.) The eyelid is next turned out to its normal position and the suture adjusted and tied with the knot in the ventral position, or farthest removed from the eyelid. The suture is allowed to remain for a week, after which it is removed.

The above operation was performed upon two foals, a Percheron and Belgian. The former had double entropion. The standard operation was done on one eye as a matter of comparison. Healing took place in all three cases in about one week. Two weeks after the sutures were removed, it was nearly impossible to detect that any operation had been performed, as the skin had contracted, leaving a normal appearance.

This method gave equally as good results in correcting entropion in foals as the standard operation and, because it is simpler and easier to perform, should be preferred by the practitioner. Because this is the common method of operation on entropion in young lambs, it was tried on foals.

COCCIDIOIDAL GRANULOMA IN CATTLE IN COLORADO*

By GEO. W. STILES, JR., M. S. SHAHAN *and* C. L. DAVIS
Pathological Division, Bureau of Animal Industry
U. S. Department of Agriculture†

Following Wernicke,[1] who, in 1892, discovered the etiological organism of coccidioidal granuloma in a skin affection of a Brazilian soldier, Rixford,[2], in 1894, in California, reported the first case in the United States. According to the California Department of Public Health,[3] there were 286 human cases on record prior to June 1, 1931, 89.5 per cent having originated in that State. Ten other states in the United States had presented one

*Received for publication, April 24, 1933.
†The authors are attached to the branch laboratory located at Denver, Colo.

or more cases, and three apparently had originated in Colorado. Both Wernicke and Rixford believed the organism to be a protozoön but Ophüls' work[4] and other subsequent studies have amply demonstrated that it is a mold.

The Disease in Animals

Giltner[5] reported the first cases of coccidioidal granuloma in cattle in 1918. He demonstrated the fungus coccidioides* in lesions from slaughtered cattle forwarded from an abattoir in San Diego, California. The lesions were confined to the bronchial and mediastinal lymph-nodes, as contrasted with the skin lesions and organic generalizations so often described in man. His studies[6] indicated that the disease was by no means of uncommon occurrence in California cattle. The subsequent 18 similar cases in cattle and one case in a sheep reported by Beck,[7] Traum,[8] and Beck, Traum and Harrington[9] have tended to entrench such a belief.

At the present time there is no record of the involvement of tissues other than the thoracic lymph-nodes in the spontaneous cases in animals. From the location of the lesion, it is believed the infection takes place through the respiratory tract, although some writers do not exclude the possibility of alimentary infection, the fungus probably reaching the lymph-nodes by way of the lymph-stream. Although there is no evidence at present of infection by way of the skin, such an avenue does not seem impossible, in view of the apparent association of some of the infection in man with soil contact. The condition, to our knowledge, has not been heretofore reported in animals outside of California.

Colorado Case (Bovine)

The case herein reported occurred in a fat, two-year-old heifer from a feed-lot in northern Colorado. Complete details of the individual's history are not available, but the information at hand indicates that she was a native-bred animal. Upon slaughter in the Denver yards, on July 14, 1931, the animal was examined by one of us (C. L. D.), who observed the posterior mediastinal lymph-node to be diseased. Macroscopically the gland was enlarged and, upon incision, displayed several encapsulated areas 3

*Ernest C. Dickson, on page 8 of the California Department of Public Health Special Bulletin 57, states, "Because of the confusion in the nomenclature, we are terming this mold 'fungus coccidioides' until it is placed in the proper classification according to class, family and genus." We concur in this temporary choice of name.

to 8 mm in diameter. The tissue was traversed by numerous trabeculous striations which gave a honeycomb appearance to the surface of the section. Upon pressure, the pockets exuded a pale, yellowish, tenacious pus, granular in consistency, somewhat resembling actinomycotic material and not entirely unlike that of tuberculosis.

The organisms were demonstrated in cover-slip preparations of the pus. They appeared as spherical bodies varying in diameter from 10 to 40μ, with a double-contoured capsule and containing a granular protoplasm. Some of the larger forms contained small spheres suggesting daughter cells. Cultures of a septate mold, uncontaminated, readily grew on meat-infusion agar and other ordinary media, and guinea pigs were infected by intraperitoneal inoculation, of both the original material and cultures. The lesions in guinea pigs somewhat resembled those of tuberculosis.

Cultures of the mold and lesions produced by it in guinea pigs were forwarded to the Pathological Division of the Bureau of Animal Industry at Washington, and the diagnosis of fungus coccidioides was confirmed.

COMMENT

This case is reported not only because of the interest in its incidence in Colorado, but with the hope that it may encourage veterinarians in this country, whether at postmortem or in the laboratory, to exercise due vigilance in their observation and study of lymph-gland lesions in cattle. A lesion atypical of actinomycosis or tuberculosis, or even typical in suspicious instances, may well be subjected to laboratory study, constantly bearing in mind the severity of the disease in man and the probable dangers from lesions or cultures.

REFERENCES

[1] Wernicke, R.: Ueber einen Protozoenbefund bei Mycosis Fungoides. Cent. f. Bakt., Orig. Bd. xii (1892), 24, pp. 859-861.

[2] Rixford, E.: A case of protozoic dermatitis. Occid. Med. Times, viii (1894), 5, p. 326.

[3] . . . . .: Calif. Dept. Pub. Health Spec. Bul. 57 (1931).

[4] Ophüls, W.: Further observations on a pathogenic mould formerly described as a protozoön *(Coccidioides immitis, Coccidioides pyogenes)*. Jour. Exp. Med., vi (1905), 4/6, pp. 443-485.

[5] Giltner, L. T.: Jour. Agr. Res., xiv (1918), 12, p. 533.

[6] Idem: Unpublished notes.

[7] Beck, M. D.: Proc. Soc. Exp. Biol. & Med., xxvi (1929), p. 534.

[8] Traum, J.: Report on transmissible diseases of cattle. Jour. A. V. M. A., lxxv (1929), n. s. 28 (4), pp. 477-480.

[9] Beck, M. D., Traum, J., and Harrington, E. S.: Coccidioidal granuloma: occurrence in animals—reference to skin tests. Jour. A. V. M. A. lxxviii (1931), n. s. 31 (4), pp. 490-499.

BOVINE OBSTETRICS AND A MOTOR TRUCK*

By S. T. MILLER, *Wenatchee, Wash.*

I would like to relate a little experience I had with a case of obstetrics in a polled Hereford heifer two years old.

There is nothing strange about the abnormal presentation, as every veterinarian has had experience with cases showing one front leg and the head out, with one leg back. This was the experience of a farmer living about 100 miles away, who called me by 'phone, on April 14, at about 2 p. m. He said that neither he nor his neighbors could "pull" the calf or find the other front leg. He wanted my advice. I told him to call a veterinarian as I was inspecting meat and it was impossible for me to leave until after 5 p. m., and I did not care to be out all night. He asked me if I could take the calf away if he would bring the cow down to me on the truck. I agreed to do so.

About 9 p. m., he arrived with the cow on the truck. I soon was at my job and in fifteen minutes he was on the road home. The trip takes about three hours of fast driving through mountains and valleys, but he had prepared the truck with a good bed of straw and covered the truck with canvas to protect the cow from the cold night air.

This is the first time in my veterinary experience (since 1892) that a case of obstetrics in a cow has been brought to me for delivery. Considering the long distance, I think perhaps there have been very few such cases on record.

Cornell Conference Dates Fixed

Dr. W. A. Hagan, dean of the New York State Veterinary College at Cornell University, has announced that the 1934 Veterinary Conference will be held on January 11 and 12. Early action in deciding upon and announcing the date for this important event was prompted by a desire to avoid conflicts with other veterinary meetings in the East, usually held at about the same time, particularly those in Pennsylvania, New Jersey and Maryland.

Wife (reading newspaper): "It says here that a well-known poet always recites his verses to his dog."

Husband: "Well, I suppose the S. P. C. A. can't be everywhere at once." —*Our Dumb Animals.*

*Received for publication, April 24, 1933.

LE RÔLE DE L'ALEXINE DANS LE RENFORCEMENT DE LA VIRULENCE MICROBIENNE (The Rôle of Alexin in Augmenting the Virulence of Microörganisms). Jules Bordet and Ernest Renaux. Ann. de l'Inst. Past., xlix (1932), 5, pp. 539-544.

Individual microbes inhabiting an inoculated medium are not entirely identical. In general the virulence increases by passages. *In vivo,* those least resistant to the defensive forces are eliminated, with resulting purification of the injected culture. The least vulnerable organisms, that is, the more dangerous ones, persist. They escape leucocytosis and thus make up an aggregate of extreme virulence.

The author already demonstrated this fact for streptococci in 1896 and later others found the same to be true for the bacillus of anthrax, the pneumococcus, etc. Inoculations made into the peritoneal cavity of laboratory animals show, an hour or two later, that certain ones among the organisms have become encapsulated with a film that resists the action of leucocytes even when the latter exist in large numbers. Others not thus coated are captured and destroyed in the leucocytic protoplasm. It seems probable that the coating consists of carbohydrate, since the color obtained by staining resembles that obtained on gelose.

Escaping the leucocytes, the coated microbes multiply without hindrance and therefore propagate descendants possessing a high degree of aptitude for taking the protective film. They thus acquire a pronounced exacerbation of virulence.

Working with *B. coli,* the authors show that humoral bactericides also suppress the more vulnerable organisms. Cultures of this organism, as do also others, contain two varieties; smooth and rough. As is well known from microbes presenting these two varieties, the general conclusion has been drawn that the smooth type is more pathogenic than the rough type. In 1912, Eisenberg demonstrated that it was possible to select germs of unequal virulence from the same cultures of *B. anthracis,* and Manniger (1919) and DeKruif (1921) demonstrated the same fact in chicken cholera and rabbit septicemia, respectively. The

difference in virulence of the two types (smooth and rough) is considerable, the rough type being very sensitive to the bactericidal action of the serum alexin, while the smooth shows pronounced resistance. The point emphasized is that pathogenic bacteria *in vivo* increase in virulence through elimination of the weak, whether the elimination results from the engulfing action of phagocytes or the bacterial action of alexin. The methods of arriving at these conclusions are described.

L. A. M.

DIAGNOSTIC DE LA TYPHO-ANÉMIE INFECTIEUSE DE CHEVAL (The Diagnosis of Infectious Anemia of the Horse). Paul Rossi. Rev. Vét., lxxxiv (1932), pp. 547-565.

The incidence of infectious anemia of the horse is increasing. The work done on the subject since the war furnishes a long bibliography.

A rational and successful campaign against the disease reposes on formal and early diagnosis which, except in regions where it is well known, is not an easy matter. The first cases may be overlooked. In making early diagnoses, clinical and necroscopic evidence are less significant than hematology, serology, inoculation of laboratory animals, inoculation of horses, etc. The virus of infectious anemia brings profound alterations to the plasma and cells. Opposing opinions in this regard are perhaps more apparent than real, being very certainly due to types of the disease which differ only in their evolution. The blood lesions of an acute case, for example, differ from those of a chronic one, in the aspect of the plasma and serum, the number and form of the corpuscles, the hemoglobin index, etc. The blood picture, therefore, may not furnish pathognomonic evidence and the same seems to apply to blood reactions (agglutination, deviation of complement, lipoido reaction, reaction of Fulton).

The author points out the facts concerning inoculation of laboratory animals (rabbits, guinea pigs, chickens, pigeons) as a means of confirming a diagnosis, and concludes that inoculation of horses is the surest method. One must be absolutely certain, however, that the test animal is not a carrier of the virus and that a remission from a natural attack is not mistaken for a reaction from the inoculation. Schalk and Roderick (*Agr. Exp. Sta., North Dakota, Agr. Coll. Bul.*, 168, Aug., 1929) followed for 14 years an experimentally inoculated horse whose blood remained virulent although the horse showed no signs of anemia. Others have shown the inconstancy of experimental inoculations

of virulent blood or blood-serum into susceptible animals. The virus may be spare or weak. In general, however, the most serious fault of this method is the high cost of the test animal.

The author reviews the various methods that have been employed to "reactivate" the symptoms as means of making a positive diagnosis. Among these are copious bleedings, injections of chemicals or heterologous serums, mallein and tuberculin. All of these have failed.

In short, there is no absolutely certain method of diagnosing infectious anemia. The practitioner must draw conclusions from the various laboratory methods available and by eliminating the presence of other diseases.

L. A. M.

La Fievre Ondulante d'Origine Bovine a Brucella Melitensis (Undulant Fever of Bovine Origin Due to *Brucella Melitensis*). Gilles, Péré and Gulty. Rev. Gén. de Méd. Vét., xli (1932), 488, pp. 476-480.

Undulant fever of bovine origin is not always synonymous with undulant fever due to the bacillus of Bang. The cow can be infected with *Br. melitensis* and transmits that organism to man. The point is illustrated by the following observation:

In a monastery where there were 27 cows, 150 sheep and 60 hogs, two caretakers and one cheesemaker were stricken with undulant fever after having been exposed to aborting cows affected with retained afterbirths. Sero-agglutination tests made on twelve of the cows, five of the hogs, and six of the sheep, selected more or less at random, gave five positive reactions (at 1:130 and 1:160) among the cows but none among the other animals. The diagnosis was confirmed further by inoculating guinea pigs with milk from the reacting cows. Four of the guinea pigs were infected and in two of them organisms of the species Brucella were isolated, and identified as *Br. melitensis* by Huddleson's method. By the same method, organisms isolated from two of the sick men proved to be of the melitensis variety. The authors conclude that here were three cases (striking examples) of undulant fever of bovine origin, occasioned by *Br. melitensis*.

It was not possible to determine whether the cows were infected by an unrecognized infection among the sheep or by a bull recently introduced into the herd. The article contains all of the details from which these conclusions were drawn.

L. A. M.

L'ECZEMA, SES RELATIONS AVEC L'ANAPHYLAXIE ET L'INSUFFISANCE HEPATIQUE (Eczema, Its Relations with Anaphylaxis and Hepatic Insufficiency). F. Maignon. Rec. de Méd. Vét., cviii (1932), 12, pp. 721-735.

Eczema is a group of cutaneous affections whose delimitation is artificial and not very precise. The causes are multiple and extremely varied, external and internal, comprising mechanical, chemical, physical, parasitic and microbian agents. For this reason eczema is not entitled to a name designating it as a morbid entity, according to many authorities. Nevertheless, the lesions of the affection so designated do have a common anatomo-pathology. In all cases, moist or dry, there is a spongiosis of the bodies of Malpighi that is preceded by a subepidermal vesiculation, characterized by infiltration of these bodies. The lesion, in effect, consists of edema with vaso-dilatation and mononuclear infiltration. Darier defines eczema as an epidermo-dermatitis manifested by sponginess of the Malpighian bodies. It is in the matter of cause that eczema is variant.

The author enumerates the various causes capable of provoking an eczema of external origin (scratching, cold, heat, sunlight, x-rays, medicinal applications, parasites, microörganisms, etc.). These, in a long discourse covering the subject in great detail, are considered in their relation to anaphylaxis. Attributing eczema of external origin merely to the irritation of the causal agent is "vague language," declares Professor Maignon. Dead and degraded proteins remaining enclosed with the living protoplasm act as sensitizing agents. The sensitization, in other words, is due to the products of disintegration of the tissues subjected to irritation (Jadassohn). In the parasitic and microbian forms it is not the violence of their toxins that causes the dermatosis but the proteins of their dead bodies.

Hepatic insufficiency can be invoked in explanation of eczema and urticaria of alimentary origin. It is especially instrumental when proteins insufficiently fragmented over-run the liver in large quantities. Digestive troubles, abnormal fermentation resulting in the production of toxic degradation products, and insufficiency of the proteolytic and antitoxic functions are likewise agencies to incriminate.

Logically, to cure eczema of alimentary origin, it is sufficient to correct the hepatic insufficiency.

The author reviews facts on opotherapy, demonstrating that extracts from organs of healthy animals do restore the nutritive

and functional activity of the corresponding organs of the living subject. The substances should be obtained by a method of extracting diastase, and they act as diastases and not as hormones, that is to say, not as excitants, which is shown by the fact that they do not act on healthy subjects and that in the sick their action is not in proportion to the dose. Their action is specific for the organ concerned.

In this form of eczema, extract of liver tissue, given to 62 animals, gave 55 recoveries with complete disappearance of the cutaneous lesions and regrowth of the hair. Twenty-seven of these showed no relapses after two years, and relapses occurring in the other 28 promptly responded to the same treatment. The seven cases in which the treatment failed were obviously affected with eczema of external origin.

The conclusion drawn is that eczema in the dog generally is due to hepatic insufficiency, and that while this dermatosis has multiple causes its pathogenesis otherwise is relatively unique.

L. A. M.

Uber den Keimgehalt der Fruchtwässer und Föten gesunder Pferde und Rinder (A Study of the Bacterial Content of the Amniotic Fluid and Feti of Healthy Horses and Cattle). Herbert Dubovsky. Tierärzt. Rdsch., xxxviii (1932), 27, pp. 459-463.

This material was secured from a slaughter-house. There were 41 equine and 35 bovine uteri, which were gravid and apparently healthy. These were obtained immediately after slaughter. The duration of pregnancy in the horse was from 20 to 47 weeks, and in the cattle from 18 to 39 weeks. Most of the uteri were brought to the laboratory for examination. Some of the larger ones were cultured immediately at the slaughter-house. The organs were examined for gross pathologic changes. None were observed. The amniotic fluid was cultured carefully and various organs from the fetus were cultured likewise. No growths were obtained. The intestinal tracts and the parenchymatous organs of the 76 feti also proved to be germ-free. The author ran tests to determine the bactericidal properties of the amniotic and allantoic fluids of both horses and cattle. He found that these fluids were inhibitory to the growths of *Staphylococcus pyogenes* and *Streptococcus pyogenes*, but the medium which was enriched with these fluids (amniotic and allantoic) showed a distinct advantage for the cultivation of *Eschcerichia coli*.

M. D. K.

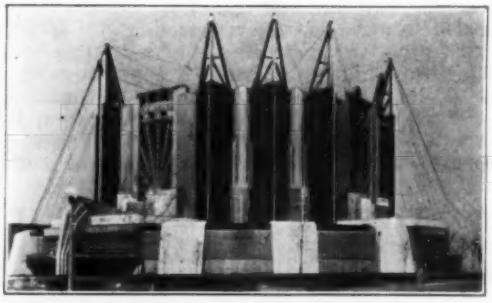
AVIAN TUBERCULOSIS IN CATTLE IN GREAT BRITAIN. F. C. Minett. Jour. Comp. Path. & Therap., xlv (1932), 4, p. 317.

The natural occurrence of avian tuberculosis in cattle in Great Britain is reported. The nature of the infection was established in three calves and in two adult bovines, belonging to three herds, but it was probable that other cases among calves existed in at least one of these herds. In two of the herds tuberculosis was proved to exist among the fowls, and these had free access to the cattle. In four instances the organisms were isolated from glands of the alimentary tract and in the fifth instance from the apparently normal intestinal mucous membrane.

Four of the five cases were detected as a result of reactions to the intradermal johnin test, but, where tests were made, the animals reacted still more strongly to avian tuberculin and in a smaller degree to mammalian tuberculin. Postmortem examination, however, failed in all cases to show the presence of bovine tuberculosis or Johne's disease. With two animals strong reactions to avian tuberculin or johnin could be elicited for a period of six months after removal from the source of infection, but at the time of slaughter one of these had apparently overcome the infection.

The strains of avian tubercle bacilli from the calves were highly virulent for rabbits and fowls; those from two adult cattle were of low virulence, probably owing to a more prolonged residence in mammalian tissues.

The practical importance of avian tuberculosis in cattle is discussed.



TRAVEL AND TRANSPORT BUILDING
CENTURY OF PROGRESS EXPOSITION, CHICAGO

Regular Army

First Lieutenant Stanley M. Nevin is relieved from further assignment and duty as student, the Cavalry School, Ft. Riley, Kan., effective upon completion of his present course of instruction, and will report to the commanding general, Fort Riley, Kan., for duty.

Major Raymond T. Seymour is relieved from further assignment and duty at the purchasing and breeding headquarters, Lexington, Ky., and from additional duty at the University of Kentucky, Lexington, Ky., effective on or about June 20, 1933, and will proceed to Fort Robinson, Neb., and report to the commanding officer, Robinson Quartermaster Depot, for duty.

Major Gerald W. Fitz Gerald is relieved from duty in the office of the Surgeon General, Washington, D. C., effective in time for him to sail on transport scheduled to leave New York City on or about June 20, 1933, for San Francisco, Calif., and upon arrival to proceed to the Presidio of Monterey, Calif., for duty.

Major Raymond A. Kelser is relieved from assignment and duty at the Army Medical Center, Washington, D. C., and from additional duty at Fort Myer, Va., effective on or about August 1, 1933, will then proceed to Boston, Mass., take station, and report to the commanding officer Boston Quartermaster Depot for duty, and to the commanding general 1st Corps Area for additional duty at his headquarters and as attending veterinarian with the Reserve Officers' Training Corps Unit, Harvard University, and at Fort Devens, Mass.

Major Francois H. K. Reynolds is relieved from assignment and duty at Ft. Sam Houston, Tex., and from additional duty at the 8th Corps Area Laboratory, effective July 1, 1933, will then proceed to Washington, D. C., and report to the commanding officer, Army Medical Center, for duty.

Major Irby R. Pollard is relieved from duty at the Robinson Quartermaster Depot, Fort Robinson, Neb., effective July 25, 1933, will then proceed to Ft. Meade, S. Dak., and report for duty.

Major Herbert K. Moore is relieved from duty at Ft. Francis E. Warren, Wyo., effective in time to comply with this order, will proceed to San Francisco, Calif., and sail on the transport scheduled to leave that port for New York, N. Y., on or about August 1, 1933; upon arrival in New York City will proceed to Washington, D. C., and report to the commanding officer, Army Medical Center, for duty for the purpose of pursuing a course of instruction at the Army Veterinary School.

Veterinary Reserve Corps

New Acceptances

Dunlap, Leo Walter..2nd Lt.....R.F.D. 7, Circleville, Ohio.

Promotions

To

Endsley, William Luther, Lt. Col....208 Live Stock Exch. Bldg., Fort Worth, Tex.

K. S. C. Prizes

The tenth annual Recognition Day program of the Kansas State College took place in the College Auditorium, Manhattan, May 5, 1933. On this occasion all winners of student prizes in the College received their awards. Names of the winners of the prizes offered the students of the Division of Veterinary Medicine are as follows:

General Proficiency

Prizes offered by Dr. E. A. Schmoker (K. S. C. '17), of Seattle, Wash.
First Prize ($7.50).....Richard Duncan Turk, '33
*Second Prize ($3.75).....Harlow Kenyon Hudson, '33
*Second Prize ($3.75).....William H. Lindley, '33

Therapeutics

Prizes offered by Jensen-Salsbery Laboratories, Kansas City, Mo.
First Prize ($7.50).....Bradbury Bedell Coale, '34
*Second Prize ($3.75).....Paul Edmond Chleboun, '34
*Second Prize ($3.75).....Carl William Schulz, '34

Pathology

Prizes offered by Dr. O. M. Franklin (K. S. C. '12), of Amarillo, Tex.
First Prize ($10.00).....Richard Duncan Turk, '33
Second Prize ($5.00).....William H. Lindley, '33

Physiology

Prizes offered by Dr. Hathan D. Harwood (K. S. C. '18), of Manhattan, Kan.
*First Prize ($5.50).....Oscar Frederick Fisher, '35
*First Prize ($5.50).....Edgar William Millenbruch, '35
Second Prize ($4.00).....Donald Clifford Kelley, '35

Veterinary Medicine

Prize of $10.00 offered by *Veterinary Medicine*, of Chicago, Ill., for the best veterinary article submitted by a K. S. C. student.
Eugene W. Peck, '33.

In addition to the prizes in the Division of Veterinary Medicine, the following undergraduates were elected to membership in Gamma Sigma Delta, the honor society of agriculture: Elmer Fred Finke, Harlow Kenyon Hudson, William H. Lindley, Richard Duncan Turk.

*Prizes divided.

Regular Army

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Major Gerald W. Fitz Gerald is relieved from duty in the office of the Surgeon General, Washington, D. C., effective in time for him to sail on transport scheduled to leave New York City on or about June 20, 1933, for San Francisco, Calif., and upon arrival to proceed to the Presidio of Monterey, Calif., for duty.

Major Raymond A. Kelser is relieved from assignment and duty at the Army Medical Center, Washington, D. C., and from additional duty at Fort Myer, Va., effective on or about August 1, 1933, will then proceed to Boston, Mass., take station, and report to the commanding officer Boston Quartermaster Depot for duty, and to the commanding general 1st Corps Area for additional duty at his headquarters and as attending veterinarian with the Reserve Officers' Training Corps Unit, Harvard University, and at Fort Devens, Mass.

Major Francois H. K. Reynolds is relieved from assignment and duty at Ft. Sam Houston, Tex., and from additional duty at the 8th Corps Area Laboratory, effective July 1, 1933, will then proceed to Washington, D. C., and report to the commanding officer, Army Medical Center, for duty.

Major Irby R. Pollard is relieved from duty at the Robinson Quartermaster Depot, Fort Robinson, Neb., effective July 25, 1933, will then proceed to Ft. Meade, S. Dak., and report for duty.

Major Herbert K. Moore is relieved from duty at Ft. Francis E. Warren, Wyo., effective in time to comply with this order, will proceed to San Francisco, Calif., and sail on the transport scheduled to leave that port for New York, N. Y., on or about August 1, 1933; upon arrival in New York City will proceed to Washington, D. C., and report to the commanding officer, Army Medical Center, for duty for the purpose of pursuing a course of instruction at the Army Veterinary School.

Veterinary Reserve Corps

New Acceptances

Dunlap, Leo Walter..2nd Lt.....R.F.D. 7, Circleville, Ohio.

Promotions

To

Endsley, William Luther, Lt. Col....208 Live Stock Exch. Bldg., Fort Worth, Tex.

K. S. C. Prizes

The tenth annual Recognition Day program of the Kansas State College took place in the College Auditorium, Manhattan, May 5, 1933. On this occasion all winners of student prizes in the College received their awards. Names of the winners of the prizes offered the students of the Division of Veterinary Medicine are as follows:

General Proficiency

Prizes offered by Dr. E. A. Schmoker (K. S. C. '17), of Seattle, Wash.
First Prize ($7.50).....Richard Duncan Turk, '33
*Second Prize ($3.75).....Harlow Kenyon Hudson, '33
*Second Prize ($3.75).....William H. Lindley, '33

Therapeutics

Prizes offered by Jensen-Salsbery Laboratories, Kansas City, Mo.
First Prize ($7.50).....Bradbury Bedell Coale, '34
*Second Prize ($3.75).....Paul Edmond Chleboun, '34
*Second Prize ($3.75).....Carl William Schulz, '34

Pathology

Prizes offered by Dr. O. M. Franklin (K. S. C. '12), of Amarillo, Tex.
First Prize ($10.00).....Richard Duncan Turk, '33
Second Prize ($5.00).....William H. Lindley, '33

Physiology

Prizes offered by Dr. Hathan D. Harwood (K. S. C. '18), of Manhattan, Kan.
*First Prize ($5.50).....Oscar Frederick Fisher, '35
*First Prize ($5.50).....Edgar William Millenbruch, '35
Second Prize ($4.00).....Donald Clifford Kelley, '35

Veterinary Medicine

Prize of $10.00 offered by *Veterinary Medicine*, of Chicago, Ill., for the best veterinary article submitted by a K. S. C. student.
Eugene W. Peck, '33.

In addition to the prizes in the Division of Veterinary Medicine, the following undergraduates were elected to membership in Gamma Sigma Delta, the honor society of agriculture: Elmer Fred Finke, Harlow Kenyon Hudson, William H. Lindley, Richard Duncan Turk.

*Prizes divided.

Phi Zeta Initiates

The annual initiation ceremonies of Gamma Chapter of Phi Zeta, national veterinary honor society, were held at the Memorial Union, Iowa State College, May 16, 1933.

Dr. R. E. Buchanan, dean of the Graduate College, head of the Department of Bacteriology and co-author of the well-known textbook, "Veterinary Bacteriology," was made an honorary member. Dr. N. O. Bevins, chief of the Division of Animal Industry, Iowa Department of Agriculture, had been elected to honorary membership and had accepted. However, he died suddenly from heart failure a few days before the initiation ceremonies were held. By unanimous vote of the Chapter a posthumous honorary membership was conferred upon Dr. Bevins, who was an alumnus of Iowa State College, class of 1911.

The following active members were initiated from the faculty: Drs. T. S. Leith, I. A. Merchant, R. A. Runnells, E. A. Hewitt, S. H. McNutt and H. E Biester. Seven members of the senior class were elected active members as follows: T. F. Bartley, L. C. Tekse, D. O. Kitchen, C. J. Sinek, J. W. Chenoweth, M. Luinstra and R. F. Estes. Six members from the junior class were initiated: W. A. Winslade, E. E. Thompson, R. P. Link, J. H. Whitlock, C. A. Pollman and F. Wilhite.

Dr E. A. Benbrook, president of the Chapter, conducted the ceremonies. A banquet was then enjoyed, followed by several informal talks. Dr. C. H. Covault was elected president for the ensuing year and Dr. Geo. R. Fowler as secretary-treasurer. W. A. Winslade, the junior student with the highest scholarship standing, was automatically made vice-president.

Errata

In the paper "Field Observations on Erysipelas in Swine Herds," by C. F. Harrington, published in the March, 1933, issue of the JOURNAL, the body temperature given in the last paragraph, page 495, should be 106° F.

In the editorial on the discontinuance of the veterinary course at the Georgia State College of Agriculture, in the May, 1933, issue, page 690, the number of veterinary graduates was given incorrectly as 49. This should have been 59. The first class, consisting of four men, was graduated in 1921, instead of 1922, as stated.

RALPH M. BUFFINGTON

Major Ralph M. Buffington, U. S. Army, retired, died at Country Club Hills, Cherrydale, Va., on April 10, 1933. He was born in Berkshire, N. Y., January 12, 1880. He was graduated from the New York State Veterinary College, Cornell University, in 1905, and immediately entered the service of the U. S. Bureau of Animal Industry. He was assigned to meat inspection at Kansas City and, a short time later, was transferred to quarantine work at the same station. In 1908 he was transferred to meat inspection at Buffalo, and the following year to Raleigh, N. C., on tick eradication. Dr. Buffington resigned from the Bureau service, September 15, 1910. He entered Army service as a veterinarian of cavalry on September 29, 1911, and upon the organization of the Veterinary Corps was appointed as a second lieutenant, June 3, 1916, attaining the grade of major on September 29, 1925. He was retired for disability in line of duty on September 30, 1930.

After returning from service with the A. E. F., France, Major Buffington served as Assistant Professor of Military Science and Tactics, in charge of the Veterinary Unit, Reserve Officers' Training Corps, Cornell University, for a period of four years, and later served as Division Veterinarian, 2nd Division, Fort Sam Houston, Tex. He was on duty at Fort Myer, Va., at the time of his retirement.

Major Buffington joined the A. V. M. A. in 1920. He was a member of Beta Chapter of Alpha Psi Fraternity,

Funeral services were held in Fort Myer chapel followed by burial, with full military honors, in Arlington National Cemetery, April 12, 1933. He is survived by his widow and one son.

W. GEORGE TURNER

Col. W. George Turner, U. S. A., retired, died suddenly at his home, Topside Farm, Hickman Mills, Mo., April 28, 1933, and was buried at Fort Leavenworth, Kan., on April 30.

Born at Port Huron, Mich., December 16, 1870, Col. Turner attended grade and high schools and took a business college course. He studied veterinary medicine at the Ontario Veterinary College, from which institution he was graduated in 1891.

With the exception of one year, Col. Turner served as a veterinarian in the Quartermaster Department, from July 1, 1893, to June 3, 1916, at which time he was commissioned as a major, attaining the rank of colonel on June 14, 1920. He served as chief of the Veterinary Corps in the Surgeon General's office at Washington, D. C., from May 10, 1926, to May 10, 1930, and was retired for disability in line of duty October 31, 1931.

A great amount of Col. Turner's service was as an inspector with animal purchasing boards, in which capacity he was considered an authority. Col. Turner served one tour of foreign service in Panama and one in the Philippine Islands.

Col. Turner joined the A. V. M. A. in 1919 and was elected second vice-president at the convention held in 1927. He served as a member of the Committee on History, 1926-28. He is survived by his widow, one son and one daughter.

NOEL ORSIN BEVINS

Dr. N. O. Bevins, of Hawkeye, Iowa, aged 44, died suddenly at the home of Dr. H. N. Strader, Marion, Iowa, May 11, 1933. Dr. Bevins had stopped in Marion, on his way from Des Moines to Cedar Rapids, to attend the monthly meeting of the Linn County Veterinary Association. A heart attack was the cause of death. He had been in good health.

A native of Hawkeye, Iowa, Dr. Bevins received his veterinary training at Iowa State College. Following his graduation in 1911, he practiced for about eleven years and in 1922 entered upon state work. On February 1, 1933, Dr. Bevins succeeded Dr. Peter Malcolm as chief of the Division of Animal Industry, Iowa Department of Agriculture.

Dr. Bevins joined the A. V. M. A. in 1924. He was a member of the Iowa Veterinary Medical Association. He is survived by his widow, one daughter, one son, his parents and one brother. Services and burial were at Hawkeye.

ALLEN O. LONGNECKER

Dr. Allen O. Longnecker died at his home in Hopkinsville, Ky., May 12, 1933. Born October 14, 1866, near Atlanta, Ill., Dr. Longnecker attended local schools and then entered the Chicago

Veterinary College. He practiced at Lincoln and Bloomington, Ill., until about 20 years ago, when he removed to Hickman, Ky. Burial was at Bloomington, Ill.

Dr. Longnecker joined the A. V. M. A. in 1916.

PERSONALS

BIRTHS

To Dr. and Mrs. H. S. MacDonald, of Toronto, Ont., Canada, a son, Donald Stewart, March 21, 1933.

To Dr. and Mrs. C. H. Haasjes, of Shelby, Mich., a daughter, Dorothy Alice, April 14, 1933.

To Dr. and Mrs. J. A. S. Millar, of Deal, N. J., a son, Harry Cecil II, April 25, 1933.

To Dr. and Mrs. E. R. Braun, of San Anselmo, Calif., a son, Robert John, April 30, 1933.

PERSONALS

Dr. Lon A. Ray (Ind. '12) has resumed practice at Cloverdale, Ind.

Dr. H. W. Broberg (K. S. C. '14) is mayor of the City of White Hall, Ill.

Dr. R. O. Mudd (McK. '19) has located at Biggsville, Ill., for general practice.

Dr. C. H. Sater (O. S. U. '02), of Hamilton, Ohio, is dog warden for Butler County.

Dr. H. W. Nobles (Ont. '02), of Coral, Mich., has been appointed acting postmaster.

Dr. F. C. Steinman (Tex. '29) has removed from Cleburne, Texas, to Columbus, Ohio.

Dr. M. M. Fletcher (Chi. '06), formerly of Illiopolis, Ill., has located in Springfield, Ill.

Dr. B. T. Buckley (Iowa '32) has located at Lennox, S. Dak., for general practice.

Dr. Charles Johnson (Chi. '20) has removed from Frederick, S. Dak., to Java, S. Dak.

Dr. C. R. Palmer (K. C. V. C. '16) gives a change of address from Lenora, Kan., to Norton, Kan.

Dr. W. H. Harlan (Colo. '29), formerly of Los Angeles, Calif., is now at Walnut Park, Calif.

Dr. G. A. Snyder (Iowa '31) reports a change in address from Perry, Iowa, to Dallas Center, Iowa.

Dr. T. G. Fultz (Iowa '10) was elected mayor of Pella, Iowa, at the recent municipal election.

Dr. Paul S. Dodd (Ind. '18), of Danville, Ill., addressed the Hoopeston Chamber of Commerce on April 3.

Dr. W. R. Anderson (Iowa '30), formerly of McCook, Neb., is now located at Worthington, Minn.

DR. J. E. HOWARD (Ont. '27) has a new location in Detroit, Mich. He is now at 846 Six-Mile Road, West.

DR. RICHARD N. SHAW (Corn. '12) has removed from Garland, Me., to Stirrup Brook Farm, Northboro, Mass.

DR. M. R. BLACKSTOCK (COLO. '10), of Spartanburg, S. C., has opened a veterinary hospital in connection with his practice.

DR. J. L. WRIGHT (U. P. '32), formerly of Greenville, Miss., has established an office at 3½ Crawford Street, Dalton, Ga.

DR. J. M. JONES (McK. '12), for the past four years state veterinarian of Tennessee, has resumed private practice at Lewisburg, Tenn.

DR. C. L. BRIGGS (U. P. '26), of DuBois, Pa., accepted a position with the Eisenberg Farm Products Company, Elizabethville, Pa., May 1.

DR. CHARLES G. HALL (O. S. U. '29), formerly with the Angell Memorial Hospital, Boston, Mass., is now located at 228 Main St., Malden, Mass.

DR. S. H. HOLLOWAY (Ont. '29) will open a new hospital for small animals at 1320 Greene Avenue, Westmount, Quebec, some time this month.

DR. O. T. HAYER (Chi. '15), of the Illinois Department of Agriculture, gave a talk on "Rabies Control" at a 4-H Club meeting at Greenville, Ill., April 21.

DR. W. A. BARNETTE (O. S. U. '13) was reëlected mayor of Greenwood, S. C., by a vote of 1,165 to 696 for his opponent, at the city election held in March.

DR. E. G. PRINGLE (Mich. '33), who has been assisting Dr. Glen Biddle (Ont. '10), of Bryan, Ohio, the past year, has started practice on his own account at Edon, Ohio.

DR. C. B. TEEL (Ind. '15) has resumed his practice at Arcola, Ill., after over three years spent in tuberculosis eradication work for the Illinois Department of Agriculture.

DR. D. A. EASTMAN (Iowa '22), of Moline, Ill., addressed the East Moline Rotary Club at the noonday luncheon, at the Short Hills Country Club, May 4. Dr. Eastman's topic was "Dogs."

DR. HARRY H. ROSS (McK. '14), of Brandon, Manitoba, is planning to attend the A. V. M. A. convention in August and is looking forward to a reunion of the 1914 graduates of McKillip Veterinary College.

DR. O. W. NELSON (Chi. '15), of DeKalb, Ill., was elected president of the DeKalb Rotary Club at the annual election, May 3. Dr. Nelson was the speaker at this meeting and the subject of his talk was "Veterinary Medicine."

DR. MARK A. QUILTY (Univ. '03), of Lake Charles, La., was elected grand commander of the Grand Commandery, Knights Templar, in Louisiana, at the 70th annual conclave of the order, held in Lake Charles, May 8.

DR. D. W. GATES (U. P. '25), who is in practice at Bellefonte, Pa., recently completed the residence requirements for the degree Master of Science at Cornell University, with a major in parasitology and a minor in pathology. The degree was conferred February 8, 1933.

DR. WM. J. LENTZ (U. P. '04), director of the Small-Animal Clinic at the University of Pennsylvania, addressed the Conestoga Veterinary Club at Lancaster, Pa., April 13. His subject was "Diseases of Small Animals."

DR. H. H. SEELY (Chi. '10), of Jerseyville, Ill., narrowly escaped serious injuries when his automobile skidded into a truck on Route 3, several miles south of Jerseyville, the latter part of April. Dr. Seely was not hurt but his car was badly damaged.

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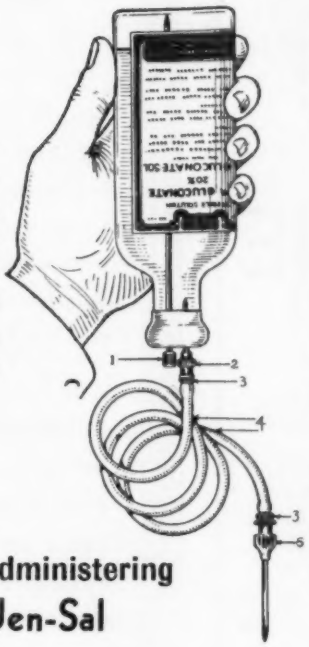

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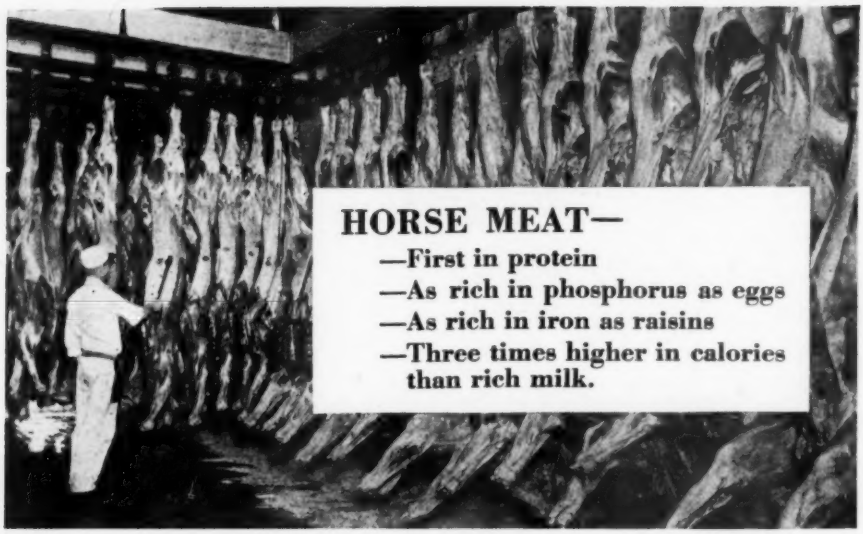
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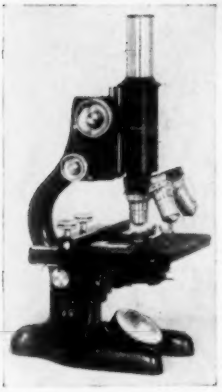

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ITS editors are Mr. Frederick Hobday, C. M. G., F. R. C. V. S., F. R. S. E., who is Hon. Veterinary Surgeon to his Majesty the King, Fellow of the Royal Society of Medicine, and the Principal of The Royal Veterinary College, London; and Mr. Gladstone Mayall, M. R. C. V. S., the author of numerous Veterinary and Agricultural textbooks.

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